THE PEOPLING OF NEW GUINEA:

CLASS I HLA AND OTHER MARKERS
A REVIEW OF THEIR CONTEXT

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Statement

This thesis describes the results of research carried out in the Human Genetics Group, the Division of Molecular Medicine, John Curtin School of Medical Research, the Australian National University, Canberra between March 1996 and May 1998.

The results presented in this thesis are my own original work, unless otherwise acknowledged.

Penelope Main

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Abstract

This study aims to address some of the issues relating to the peopling of New Guinea. The project was conducted in two parts: a review of the archaeological, linguistic and biological evidence for the peopling of New Guinea, and the DNA typing of selected New Guinea populations and New Caledonians for class I HLA (human leucocyte antigen) polymorphisms to draw inferences about the peopling of New Guinea.

Archaeological evidence suggests that people have been in New Guinea for at least 40-60,000 years. At this time New Guinea and Australia were a single land mass. Little is known about the number or nature of migrations into New Guinea or the subsequent population movement on the island although there is evidence of movement between the mainland and the Bismarck Archipelago from about 20,000 years ago. About 3,500 years ago Austronesian-speaking people arrived in the Bismarck Archipelago from where they moved further into the Pacific to people Remote Oceania and to the coastal regions of New Guinea. They were associated with many cultural changes identifiable in the archaeological record.

New Guinea is the most linguistically diverse region in the world - over 20 percent of the world's languages are spoken on the island. Austronesian languages form the largest language family on the island, being spoken in many coastal regions. The rest of the indigenous people speak a variety of non-Austronesian languages which have been categorised into about 20 unrelated phyla.

Many attempts have been made to identify a unique Austronesian marker. Although no unique Austronesian markers have been identified, in general multivariate analysis is able to separate Austronesian from non-Austronesian populations. Analysis can be complex, however, because some populations speak an Austronesian language but are genetically non-Austronesian and vice versa.

HLA genes are used in population studies because they are extremely polymorphic and show marked variation between different populations. Direct DNA typing methods for class I genes have only recently been developed and are able to detect even single nucleotide differences between alleles. This is the first major study in which they have been applied to Melanesian populations. DNA typing was carried out on eight Melanesian populations including highlanders, northern and southern highlands fringe populations, a Sepik population, north and south mainland coastal populations and populations from the Bismarck Archipelago and New Caledonia. Although serology had previously detected a number of polymorphisms in these populations, DNA typing was able to split them into several subtypes thereby providing new information about population interrelationships and migrations in this region.

The study concluded that, based on HLA and other evidence: Melanesians are likely to have evolved from the same ancestral stock as Australian Aborigines but have since differentiated into a different racial group and there are likely to have been multiple waves of migration into New Guinea. Highlanders are likely to be the descendants of earlier migrations and have been isolated for a long period of time. Northern highlands fringe and Sepik populations are likely to share a common ancestry which has since differentiated due to long term isolation and the relative proximity to the coast of the Sepik. In contrast southern fringe populations are likely to have a different origin, possibly from the Gulf region although there appears to be some admixture with neighbouring groups.

Coastal populations have a wider range of polymorphisms because they have been overlaid by later population movement along the coast from Southeast and mainland Asia that did not reach Australia or remote Oceania. Other polymorphisms detected in these populations may have been introduced by the movement of Austronesian-speaking and other more recent groups of people into the Pacific because they share many polymorphisms with Southeast Asians, Polynesians and Micronesians that are not found in highlanders or Australian Aborigines. Backmigration has occurred northward from northern Vanuatu, and into Melanesia from Polynesia and Micronesia.

Original Presentations

Oral Presentation

Has the genome adapted to malaria at the HLA locus?

Australian Society for Human Biology Conference, Canberra, 3 December 1996

Poster Presentation

HLA class I diversity in Papua New Guineans

Human Genetics Society of Australia Conference, Perth, 21-25 July 1997

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Introduction

Since their first discovery by Europeans, New Guinea populations have fascinated biological anthropologists because they offer a unique opportunity to study the evolution of human diversity. This thesis aims to throw further light on the peopling of New Guinea by reviewing literature from archaeology, linguistics and biological anthropology and by presenting original laboratory findings on the class I human leucocyte antigen (HLA) system. Issues such as the probable relationship between contemporary Melanesians, Australian Aborigines and other regional populations and the likely interrelationships between contemporary New Guinea populations are considered in this context.

The thesis is presented in two parts - the first selectively reviews what is known about the peopling of New Guinea in the context of Melanesia. The second presents the results of the laboratory component of the project which used newly developed PCR/SSO based typing protocols to investigate the complete polymorphism of HLA class I genes in eight Melanesian populations from inland New Guinea and coastal Melanesia. The HLA typing results are analysed in the context of other regional populations including Australian Aborigines, Polynesians, Micronesians and Asians. The final discussion places these findings in the context of what is already known about the peopling of New Guinea. The study is part of a larger on-going program being conducted by the Human Genetics Group, JCSMR and School of Archaeology and Anthropology, ANU.

HLA is a useful marker for population studies because it is extraordinarily polymorphic and shows marked variation in allele frequencies and linkage disequilibrium relationships between different populations. HLA, however, is subject to natural selection which may have played a significant role in shaping the HLA polymorphisms of contemporary populations. Therefore HLA data must be interpreted in the context of other available gene markers in population studies. Human genetics is able to provide a different dimension to the study of population origins and interrelationships from archaeology or linguistics.

Chapter 1 The Peopling of New Guinea in the Context of Melanesia: archaeological and linguistic evidence

1.1 Introduction

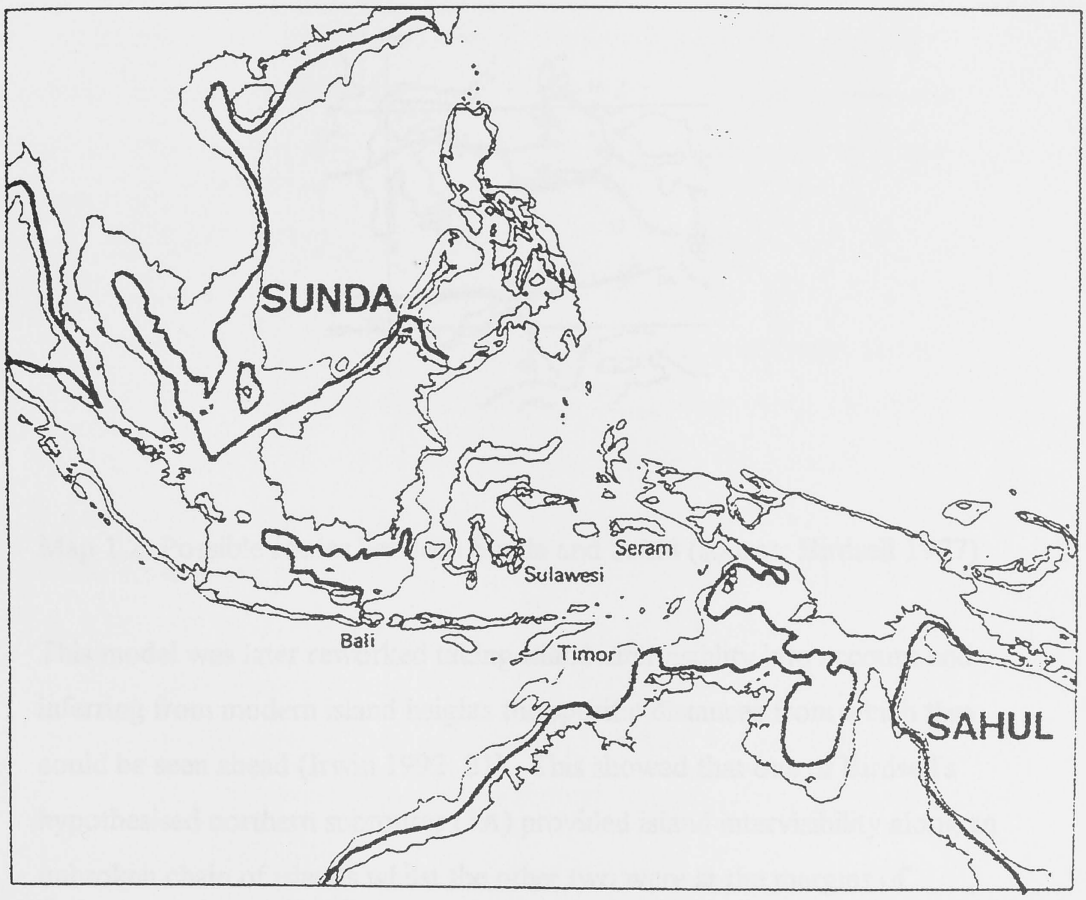
Since the earlier part of the 19th century, European scholars have divided the South Pacific into three cultural provinces: Polynesia, the many islands; Micronesia, the small islands; and Melanesia, the black islands, referring to the darker skin colour of most of its inhabitants. Culturally, linguistically and biologically, the inhabitants of Polynesia are the most homogeneous, and those of Melanesia, from New Guinea to Fiji, by far the most diverse. Reconstructions of the human prehistory of Melanesia have relied on archaeology, linguistics and biological anthropology. This chapter reviews selectively what is known of the archaeological and linguistic evidence for the peopling of New Guinea in the context of Melanesia.

1.2 Biogeography of New Guinea and Island Melanesia

During the Pleistocene, sea levels were lower than today, exposing two large continental shelves: Sahul linking New Guinea and Australia together in a single land mass; and Sunda extending mainland Southeast Asia as far as east Bali and Borneo (Map 1.1). They were separated by the Archipelagoes of Wallacea which included Sulawesi and Seram. At this time, the Bismarck Archipelago and Solomon Islands comprised several closely linked large islands, whilst further into the Pacific the islands were smaller and more remote.

Biogeographically, although New Guinea is considered to have the richest and most varied biota in the Pacific Islands, its flora, insect and bird faunas represent an impoverished subset of Indo-Malaysian genera which is

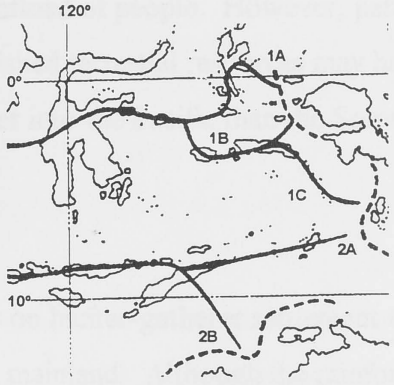
concentrated in the lowland rainforest (Green 1991). Higher, the rainforest has an increasingly Gondwanaland composition until it peters out about 4,000m. Above this altitude there is a zone of heaths and tundra, then rock, and, in places, snow and ice (Allen 1992). The faunal resources of New Guinea are basically Australian but are forest rather than open-country adapted, relatively small and often arboreal. East of New Guinea, the biota becomes markedly impoverished even in Near Oceania, the region from New Guinea through the Bismarck Archipelago to the Solomon Islands, where the islands are still relatively close together (Green 1991). The distances between islands become greater in Remote Oceania, which includes Vanuatu, New Caledonia and Fiji, and the flora and fauna becomes more attenuated.



Map 1.1: Reconstruction of the Sunda and Sahul land masses at sea levels 50 and 200 metres below present (after: Lilley 1992)

1.3 Human Settlement

The idea that founding populations reached Sahul by island hopping became widely accepted following a detailed examination of possible routes based on inter-island distance and angle of island target (Birdsell 1977). Two major routes were hypothesised, the first being via Sulawesi and Seram to the west coast of New Guinea and the second along Java, Bali, Lombok, Sumbawa, Flores and Timor to north west Australia. These were divided into five subroutes, three for the northern route and two for the southern route (Map 1.2). The maximum distance between islands was approximately 100 kilometres in Pleistocene times.



Map 1.2: Possible routes between Sunda and Sahul (source: Birdsell 1977)

This model was later reworked taking island intervisiblity into account and inferring from modern island heights the specific distances from which they could be seen ahead (Irwin 1992: 21). This showed that one of Birdsell's hypothesised northern subroutes (1A) provided island intervisibility along an unbroken chain of islands whilst the other two were at the margins of intervisibility. Both the southern routes were blind. Two-way intervisibility by land extended from mainland Asia to the end of the Bismarck Archipelago, possibly including Mussau. However, crossing to the Solomon Islands would have involved one-way intervisibility by sea, i.e. land appeared ahead before

being lost from sight behind, while voyaging to Manus would have involved a blind crossing of 60-90 km.

Irwin (1992: 22) describes the region from Southeast Asia through New Guinea to the Bismarck Archipelago as a 'voyaging corridor' sheltered from northern and southern tropical cyclones and representing a region of easy island hopping in both directions, a 'nursery' for the development of maritime technology and a safety net which allowed the first voyagers to return safely. It is unlikely that colonisation was an accidental event as seasonal changes in wind patterns would have allowed return journeying, so that the founding population of Near Oceania would probably have been larger than a single boatload of people. However, patterns of subsistence related to the impoverished terrestial resources may have limited the early spread of people further into the Pacific than the Solomon Islands (Green 1991).

There were limitations on hunter-gatherer settlement even on the better endowed New Guinea mainland. Although the rainforest environment is among the most productive on earth, faunal and plant food suitable for human consumption is not abundant or easily accessible except at the edge of the forest (Bailey et al. 1989: 60). In particular, edible plant products such as fruits, flowers and seeds are often sparse, difficult to collect and may require extensive processing. Although the total number of faunal species present in the tropical rainforest may be high, the total animal biomass is often low as there is a low numerical density for a given species (Bailey et al. 1989: 62).

1.4 Archaeological Evidence for the Peopling of Melanesia

Archaeology tells us that Sahul has been occupied from between 40,000-60,000 years before present (BP) (Spriggs 1997: 39). By 30,000 BP people had spread throughout Sahul, the Bismarck Archipelago and, shortly afterwards, the Solomon Islands. However, as a result of the difficulties of navigation and settlement discussed above, Remote Oceania was not inhabited until after about 3,000 BP by a linguistically and culturally distinct people - the Austronesians, who were responsible for the discovery and settlement of much of this part of Oceania.

The early inhabitants seem to have coped with the deficiencies of their environment by the management of forest resources (Mountain 1990). The extent to which, if at all, this management developed over time a horticultural or even agricultural aspect to the economy is a matter of debate, as are claims for the presence of pig by 6,000 BP. However, the Lapita culture associated with the appearance of Austronesians in the Bismarck Archipelago around 3,500 BP was responsible for introductions or reintroductions of the plants and animals characteristic of Pacific economies, as well as innovations in material culture, particularly pottery, though some scholars claim an earlier presence for this.

Discussion of the archaeological evidence for the peopling of New Guinea in the context of Melanesia is divided into three sections: New Guinea, subdivided into highlands and lowlands; Near Oceania, the island world of early settlement to the end of the Solomon chain; and Remote Oceania, the island world of late settlement beyond the Solomon Islands.

1.4.1 The New Guinea Mainland

1.4.1.1 The New Guinea Highlands

The term highlands in this thesis denotes those areas of the central cordillera of New Guinea above 1,000 m above sea level and should not be confused with the areas encompassed by the Papua New Guinea administrative provinces of Enga, Simbu, Southern Highlands, Western Highlands and Eastern Highlands (Lilley 1992: 150). The earliest archaeological evidence of human occupation of the highlands is provided by two sites, one located at Kosipe (26,870 ± 590 BP) (White et al. 1970) and the other at Nombe, near Chuave (27,000 ± 550 BP) (Gillieson and Mountain 1983). Of these sites, the open site at Kosipe is the more strategically placed for activities at the tree-line, whereas Nombe rockshelter has rich faunal remains of terrestrial and arboreal animals derived from a wide altitudinal range (Hope and Golson 1995).

As well as Kosipe and Nombe, other late Pleistocene sites include NFX (1550 m) in the eastern highlands (Watson and Cole 1977), Wanelek (1680m) in the Bismarck-Schrader Range (Bulmer 1977) and Yuku (1280m) at the base of the Mount Hagen Range (Bulmer 1975). Batari, in the eastern highlands, has been omitted because of reservations about the date of the initial human use of the cave (White 1972: 16).

As the weather warmed, there was a gradual increase in the number of known archaeological sites in the highlands and the density of archaeological debris within them, suggesting a population increase (Mountain 1993). There is evidence of human activity at Kosipe, Nombe and Yuku beyond the end of the Pleistocene. Newly occupied sites include Manim (1770 m) in the western highlands, Kafiavana (1350 m) in the

eastern highlands and Kiowa (1530 m) in Simbu Province. The presence of marine shells dating to 9,000 BP at Kafiavana provides strong evidence of trade links with lowland groups at this time (White 1972: 96).

Indirect evidence of early human activity in the New Guinea highlands is provided by pollen and charcoal residues. In rainforest areas forest disturbance accompanied by fire is more likely to be associated with clearance than with hunting (Flannery 1994: 295, Hope and Golson 1995: 822), although small local clearances could have been used for the exploitation of fauna (Mountain 1993). In the Baliem Valley, Irian Jaya, pollen and charcoal collected from a shallow pond on the summit of Supulah Hill (1580 m) showed that limited forest clearance and burning of a *Nothofagus* forest was occurring by 32,000 BP (Swadling and Hope 1992: 22). A further sample of charcoal occurring in slopewash deposits at the same site was dated to 28,000 BP (Haberle et al. 1991). Examples from New Guinea include a large increase in charcoal at Kosipe about 30,000 BP possibly caused by burning reed swamp for settlement (Hope 1982) and charcoal from a hearth at Kuk swamp in the Mount Hagen area with a similar date (Golson and Hughes 1977: 16).

It has been argued that agriculture developed independently in the New Guinea highlands along the Wahgi Valley from about 9,000 BP, involving gradually intensifying forest clearances associated with periodic drainage of swampland (Golson and Gardner 1990). It has not, however, been clearly established whether the site really provides evidence of agriculture before 5,000 BP, when the evidence become more compelling, or whether it represents a development of late Pleistocene plant manipulation. If it really is agriculture, the site would predate the earliest known evidence of agriculture in Southeast Asia by several thousand years (Yen 1982: 281). It is not known whether the site is based on mountain species or plants brought up from below as the climate warmed or, if the latter, even

whether the plants were indigenous or introduced from overseas by people not otherwise recognisable in the archaeological record.

Spriggs (1997: 84) argues that the Lapita culture and the range of innovations with which it is associated represents the first convincing evidence for fully developed agriculture in the region and that it spread from the Bismarck Archipelago only 2,000-3,000 years ago. However, though it appears that the domesticated animals associated with Pacific agriculture today, dogs, chickens and pigs, appeared in the region about this time, the nature and extent of the ecological transformation from forested to open landscapes recorded in the highlands is argued to require the practice of plant cultivation for a long time before this.

1.4.1.2 The New Guinea Lowlands

The oldest known coastal sites are the Huon Peninsula site previously discussed (Spriggs 1997: 39) and the Lachitu cave site in the Vanimo region of the north coast, which shows evidence of occupation dating from 35,000 BP (Gorecki et al. 1991). Waisted axes, such as those found on the Huon Peninsula, are interpreted by Groube (1989) to be evidence of forest manipulation. The earliest direct evidence for the use of tree products is the utilisation of *Canarium* nuts in the Sepik about 14,000 BP (Gorecki pers. comm. reported by Yen 1995). These nuts have been found in a wide range of sites including in the Bismarck Archipelago and Solomon Islands by 9,000 BP (Yen 1990: 262, 1995: 838-9) and were an important food resource because they grow in large numbers and can be stored for several years (Spriggs 1997: 55). A wider range of tree crops, including betelnut (*Areca catechu*), candlenut (*Aleurites* sp.) and coconut (*Cocos* sp.) was found at the Dongan midden in the lower Sepik/Ramu dating to 5,830 ± 90

BP (Swadling et al. 1991). Many of the plants identified at this site do not grow at altitudes above 1,000 metres.

Over the last 10,000 years, human intervention has significantly altered the pattern of vegetation in many lowland areas (Allen 1992). Large areas of grassland have been created in areas such as the Sepik Plains and Markham Valley, with evidence of forest disturbance in the latter from 7,500 BP (Garrett-Jones 1979). Similarly, large areas of eucalypt savanna were created on the south coast and near the mouths of major rivers (Allen 1992).

There is little data available on the prehistory of the highland fringe, except for the Jimi-Yuat area where it is likely that the valley floors of the major rivers were occupied from the early Holocene or late Pleistocene (Gorecki 1989: 184). The presence of relict grasslands in fringe areas suggests a more intensive past occupation (Gillieson et al. 1985).

The archaeology of the lowlands is distinguished from that of the highlands by the appearance of pottery attributed to immigrants or influences from overseas. There is disagreement as to whether it dates before Lapita or is a development from this. Swadling's claim for pottery at around 5,600 BP at Akari in the Sepik (Swadling et al. 1991) is weakened by discrepancies between radiocarbon dates on charcoal and shell. Bulmer's (1985: ~~before~~ 127) statement about pottery at 3,000-4,000 BP at Wanelek, a highland location in the Schrader Ranges, is inconsistent with statements she makes elsewhere about the site (Spriggs 1996: 334). This leaves Gorecki's evidence for thirty-five sherds in levels dating around 5,400 BP at the Taora rockshelter on the West Sepik coast (Gorecki et al. 1991), which Spriggs (1996: 329) argues may have come down from a higher level.

Only a couple of fragments of Lapita have been found on mainland New Guinea, but when pottery appears, as it does in the form of the so-called Papuan red-slipped ware, its spread is virtually instantaneous, like that of Lapita itself (Irwin 1980, 1991). Red-slipped pottery is first found east to west along the south coast of Papua from 1,800 BP and from 1,500 BP along the north coast and islands offshore from Madang (Lilley 1988). Like Lapita, its spread is associated with a distinctive settlement pattern and the long-distance movement of obsidian and other cultural materials. There is no doubt that the south Papuan coast pottery derives from Lapita.

1.4.2 Near Oceania

The earliest known sites in Near Oceania are: Yombon, an open site on New Britain (35,000 BP) (Pavlides and Gosden 1994); Matenkupkum (35,000 BP) (Gosden and Robertson 1991) and Buang Merabak (32,000 BP) (Balean 1989), two coastal cave sites on New Ireland which were only used sporadically until about 20,000 BP; and Kilu, a cave site on Buka (29,000 BP) (Wickler and Spriggs 1988). During the Pleistocene, the Solomon Islands chain was joined from Buka to Nggela, forming a much larger landmass than New Britain at the same time. The inland location of Yombon is testimony to the ability of the Pleistocene settlers to live in the rainforest depite the limitations discussed earlier. The two New Ireland sites give evidence of early exploitation of littoral resources. Examination of stone tools from Kilu showed residues from taro suggestive that human selection of tubers for starch had already commenced (Loy et al. 1992). Shells and marine fish bones were also present.

Matenbek (20,000 BP) (Allen et al. 1989), Panakiwuk (15,000 BP) (Marshall and Allen 1991) and Balof 1 (14,000 BP) (White et al. 1991) on New Ireland, Pamwak on Manus (13,000 BP) (Fredericksen et al. 1993)

and Missil on New Britain (11,000 BP) (Specht et al. 1981) were all occupied during the late Pleistocene. During this period there was a low-level but persistent transfer of New Britain obsidian to New Ireland from 20,000 BP; evidence of human introduction of flora and fauna from the mainland to the Bismarck Archipelago from 20,000 BP; and major discontinuities in cultural deposition between 7,000 and 10,000 BP (Spriggs 1997: 51). In the Solomons, Kilu was abandoned from 20,000 to 10,000 BP and reoccupied from 10,000 to 5,400 BP. *Canarium* nuts are found there from about 10,400 BP. Three other pre-Lapita sites are: Palandraku on Buka, dating at about 5,000 BP (Wickler 1990) and two sites on Guadalcanal dating at 6,285-4,415 BP and 4,230-3,680 BP respectively (Roe 1992).

The Lapita culture appears in the Bismarck Archipelago from about 3,500 BP as previously discussed. On reaching the islands of Near Oceania, some of the newcomers passed quickly into Remote Oceania, arriving in the area of Fiji, Tonga and Samoa by 3,000 BP. Those who stayed found themselves in a region of long prior settlement and this is likely to account for the coastal and offshore island location of their villages. Subsequent centuries saw the development of new cultural forms, archaeologically mainly expressed in the style and decoration of pottery, which may reflect the growing cultural integration of newcomers and older residents. The contribution of the newcomers and the resident populations to the eventual cultural mix is still a matter of debate among archaeologists. In the Solomon Islands there was no Lapita except on Buka due perhaps to the distribution of existing local populations throughout the rest of the chain. The Lapita phase finished about 2,500 BP and was replaced by other styles in the northern Solomons, as well as in the Bismarcks, which may reflect a cultural transformation due to the culture contact situation described above.

1.4.3 Remote Oceania

The earliest archaeological sites in Remote Oceania are associated with the Lapita complex and date from about 3,200 in Vanuatu and New Caledonia to about 3,000 in Fiji (Spriggs 1995: 116). As has already been indicated, the settlement of this region was delayed not only by the greater challenges it presented to seagoing skills and craft but also perhaps by the requirement for a well-developed agricultural economy to compensate for its impoverishment of terrestial resources.

The western Lapita substyle found in the Bismarcks after 3,200 BP is the earliest style of Lapita ware in the Solomons, Vanuatu and New Caledonia (Anson 1986). Although this style is less elaborately decorated than the earlier Far Western style found in the Bismarcks, it is still decorated with the distinctive dentate stamping (Spriggs 1995: 116). Western Lapita drops out of the archaeological record in some areas about 2,000 BP and in others by 2,500 BP. Eastern Lapita, found in Fiji and Western Polynesia from about 3,000 BP, is still simpler and has fewer vessel forms and a coarser dentate stamping. It is most closely similar to Lapita ware from northern Vanuatu and drops out of the archaeological record about 2,000 BP in Tonga and 2,800 BP in Samoa (Spriggs 1995: 116).

Throughout Remote Oceania, Lapita was replaced by different non-dentate pottery styles. In Vanuatu, there is the Mangaasi pottery tradition in which pottery was incised and had applied relief work on it (Spriggs 1997: 140). In New Caledonia, Podtanean pottery, a form decorated with carved paddle impressions developed and is found in some sites in association with Lapita ware (Spriggs 1997: 145). Podtanean pottery has been found in association with Lapita ware in some, but not all, New Caledonian sites (Sand 1995: 79-82). The clay and temper composition of the two kinds of

pottery are identical and differ only in the final stages of manufacture suggesting that they may have been made by the same potters (Galipaud 1990). Outside of New Caledonia, paddle impressed pottery is a component of Lapita sites only in Fiji and Western Polynesia in sites of this age (Spriggs 1997: 145). A third style, the Puen style, is also found in New Caledonia from 2,500 BP with a similar incised style to Podtanean pottery (Spriggs 1997: 145).

The replacement of Lapita by non-Lapita styles of pottery in Remote Oceania seems to be the replication of a process that also took place in Near Oceania as a result of the influence of newer and older cultural traditions on each other and their growing integration. This process went on as far as Fiji and the complex cultural mixing that it represents is what archaeologically constitutes Melanesia and divides it from Polynesia where the Lapita founding culture was not overlain in the same way. Note that there are parallels of ceramic development between different parts of island Melanesia, which argues for intercommunication of Lapita, which after a time broke down (Wahome 1997).

Finally, there is archaeological evidence of Polynesian back migration during the last 700 years mainly in the form of burials, cultural artefacts and oral tradition (Garanger 1982: 56) and linguistic evidence dating even earlier. Interestingly there is linguistic and cultural evidence of population movement from Micronesia into Near Oceania about 700 BP (Intoh 1996).

1.5 Linguistic Evidence for the Peopling of New Guinea

The languages of Melanesia are extremely diverse. There are nearly 1,000 languages, falling into at least twenty unrelated genetic stocks and isolates, spoken on the New Guinea mainland today and approximately another 300

languages spoken throughout island Melanesia belonging to several stocks (Grimes 1992). Most languages of Melanesia have less than 5,000 speakers, although there are exceptions including Standard Fijian (330,000), Enga (170,000) and Melpa (130,000) (Pawley 1997).

Factors contributing to the diversity of languages in New Guinea, in particular, include small polities and hostile neighbours, a long period of occupation allowing more time for local diversification and the isolation of communities by biogeographical constraints such as swamps, rainforest and mountain ranges on the mainland and ocean gaps between islands (Pawley 1997). In addition, Foley (1986) talks about cultural attitudes that view language traits as exchange items as well as symbols of unique identity.

A first division of the languages of Melanesia is usually made by contrasting the Austronesian family with all other families collectively termed 'non-Austronesian' or Papuan languages i.e. not Austronesian. Of these, only the Austronesian languages have a well established history, being associated in Oceania with the spread of the Lapita complex. In contrast, much less is known about the more numerous non-Austronesian languages, which have been in New Guinea for a much longer period.

1.5.1 The Austronesian family

Austronesian languages form the largest language family in the world in terms of numbers of languages and are scattered across a third of the globe from Madagascar, throughout much of island Southeast Asia and Oceania to Easter Island (Wurm and Hattori 1981-83). In New Guinea, they are found scattered intermittently along the north coast of the island, throughout the length of the Markham Valley, around the tip of Papua and

along the south coast for about 200 kilometres. Austronesian languages are also spoken throughout island Melanesia.

All Austronesian languages have descended from a common ancestral stock, proto-Austronesian, which may have had close links to the ancestors of several families of present day Southeast Asian languages (Reid 1994). It is likely that the primary dispersal centre of the Austronesian languages was Taiwan and they expanded into the northern Philippines around 2 500 to 3 000 BC (Blust 1995, Bellwood 1997). From the southern Philippines linguistic evidence suggests a split into two major population segments, Central-Eastern Malayo-Polynesian (CEMP) and several other groups that are located in the Philippines, Western Indonesia and Malaysia. CEMP split into two streams, Central MP which moved south into the central Moluccas and then west through the Lesser Sunda Island, and Eastern MP (EMP) which moved eastward around the north coast of New Guinea and then split into South Halmahera-West New Guinea and Oceanic, the latter moving into the Pacific where it is associated with the Lapita culture complex. The linguistic evidence indicates that the early appearance of Austronesian speakers in northwest Melanesia was the result of a single movement (Grace 1961, 1964).

Reconstructed Oceanic terminologies have been used to provide insight into the culture of Proto-Oceanic speakers and the speakers of the various interstage languages (papers cited in Pawley and Ross 1994). Proto-Oceanic speakers preserved a very high proportion of proto-Malayo-Polynesian and proto-Eastern Malayo-Polynesian terms for a wide range of cultural domains including seafaring, fishing, horticulture and pottery, implying that there was a continuity in many cultural components of life.

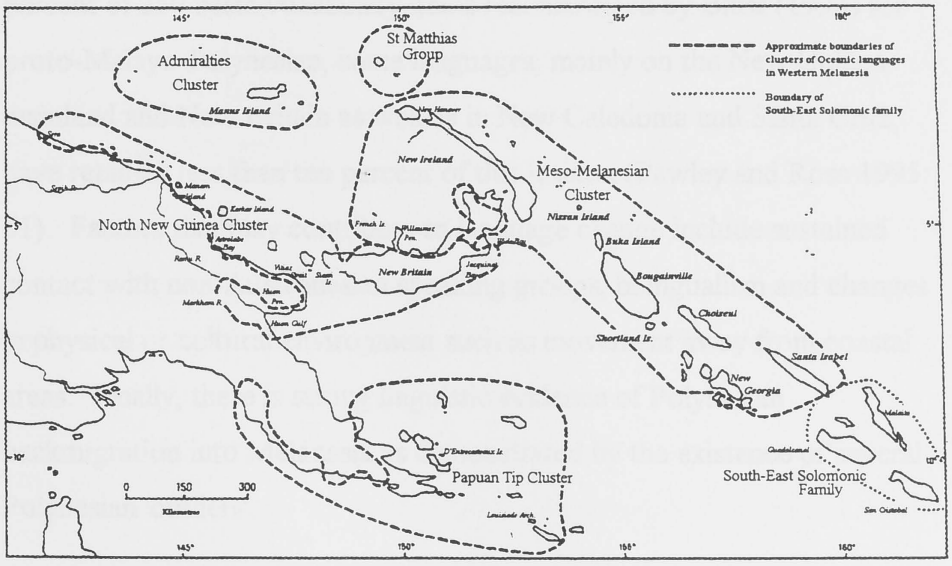
In seeking to identify the sequence of genetic splits in languages, linguists consider the patterns of innovations across languages in order to identify subgroups. Ross (1988) recognises two types of subgroups: innovation-defined subgroups and innovation-linked subgroups. In the former, all members of a subgroup exclusively share a common set of innovations, suggesting they developed from a common ancestral language. In the latter, the innovations form an overlapping pattern, reflecting the fact that the languages of the subgroup once formed a network of related dialects which later diverged.

Using these criteria, Ross recognises nine major subgroups for Oceanic: the Admiralty Islands; St Matthias Island; Western Oceanic, incorporating New Guinea, the Bismarck Archipelago and the northern Solomon Islands; Southeast Solomonic; North/Central Vanuatu; South Vanuatu; Southern Oceanic, incorporating New Caledonia and the Loyalty Islands, Central Pacific and Nuclear Micronesian. Most of these are innovation-linked subgroups (Pawley and Ross 1993). On the basis of the distribution of the subgroups, the Bismarck Archipelago is the most likely dispersal centre for Oceanic.

Western Oceanic comprises three subgroups: Meso-Melanesian, Papuan Tip and the North New Guinea cluster (Map 1.3) (Ross 1988: 25). The first two are innovation-defined language groups, whilst the latter is innovation-linked being comprised of three innovation-defined groups (Ngero/Vitiaz, Huon Gulf and Schouten). However, other innovations are shared across the groups but not between all of them. New Britain is the most likely dispersal centre for the Western Oceanic group (Pawley and Ross 1995: 58). The fact that two innovations are shared by most member languages of the widespread Western Oceanic group suggests that it descended from a dialect network which once occupied a limited area.

This area is likely to have been along the north coast of New Britain, i.e. the seam between the Meso-Melanesian and North New Guinea groups.

If this is the case, Western Oceanic speakers would have expanded in either of two directions: on the one hand, a progressive occupation of the New Britain coast, New Ireland from south to north and the northwest Solomonic area to form the Meso-Melanesian group; on the other hand, a movement across the Vitiaz Strait to the mainland coast of New Guinea followed by a split between the North New Guinea group occupying parts of the north coast of New Guinea and associated offshore islands and the Papuan Tip group. Within a short time the ancestral north New Guinea network split into the Schouten, Ngero/Vitiaz and Huon Gulf groups.



Map 1.3: Distribution of Western Oceanic languages (source: Ross 1988 25)

Linguistic evidence supports the theory of a single quick dispersal of Austronesian speakers across Melanesia into the central Pacific and Micronesia. Its association with the Lapita cultural complex discussed earlier in this chapter is well established. Archaeological dating suggests that settlement of the Papuan Tip area may not have occurred until later,

reaching Central Papua about 2,000 BP (Allen 1977a, 1977b, Bulmer 1982). The earliest archaeological evidence of Austronesian-associated culture along the north coast of the mainland at present is no earlier than about 1,500 BP (Lilley 1992). The fact that so many Oceanic subgroups form linkages rather than families suggests that within each major island group the main mode of settlement was a continuous expansion, although there are some exceptions to this pattern such as the Schouten chain, which is sufficiently different from its neighbours to imply that Proto-Schouten split from the North New Guinea dialect network fairly early (Ross 1988: 388).

Although some Austronesian languages have retained reflexes of 35-45 percent of 250 basic vocabulary items reconstructed by Blust (1993) for proto-Malayo-Polynesian, some languages, mainly on the New Guinea mainland and New Britain as well as in New Caledonia and Santa Cruz, have retained less than ten percent of this lexicon (Pawley and Ross 1995: 61). Factors that may contribute to language change include sustained contact with non-Austronesian speaking groups, bilingualism and changes in physical or cultural environment such as movement away from coastal areas. Finally, there is strong linguistic evidence of Polynesian backmigration into Melanesia as demonstrated by the existence of several Polynesian 'outliers'.

1.5.2 Non-Austronesian languages

Non-Austronesian (Papuan) languages are spoken over most of the New Guinea mainland and parts of eastern Indonesia, the Bismarck Archipelago, the Solomon Islands and Santa Cruz and predate the arrival of Austronesian speakers into Oceania. There are over seven hundred such languages, organised into some twenty unrelated families or phyla plus

several isolates (Wurm and Hattori 1981-1983). The comparative study of non-Austronesian languages has not advanced far because of inadequate data, difficulties in identifying individual language boundaries and the common use of loan words.

The largest non-Austronesian language group is the Trans New Guinea phylum (TNG), comprising almost 500 languages. This group extends along almost all of the central cordillera of the New Guinea mainland and includes most non-Austronesian languages to the south of the highlands and many to the north, as well as the non-Austronesian languages of Alor, Pantar and Timor. Although early theories regarding the unity and scope of the TNG phylum were based mainly on typology (Wurm et al. 1975), later analyses using genetic comparative techniques have confirmed the genetic unity of the core of the original TNG phylum grouping, though some modifications have been made (Pawley 1995, 1997, Ross 1995). It divides into a number of stocks, although the precise membership and subgrouping remain controversial (Pawley n.d.). Although proto-TNG has not been reconstructed as yet in detail, work has started on the historical phonology, lexicon and morphology of sub-groups within the phylum. Comparison of proto-Madang-Adelbert reconstructions with Goroka-Kainantu and Finisterre-Huon languages has yielded a reconstruction for an ancestral language to these groups, proto-Northeast New Guinea, although this may not be a closed subgroup within TNG.

Why do TNG languages have a much wider distribution than other non-Austronesian families? One suggestion is that agriculture may have provided the cultural advantage that allowed TNG speakers to spread throughout the highlands and over large parts of lowland New Guinea. Evidence of the distribution of early agricultural activity is not abundant. There is archaeological evidence from the Wahgi valley (Hope and Golson 1995: 823-834) and vegetation evidence from the Baliem valley (Hope and

Golson 1995: ~~before~~ 835) dating before 7,000 BP with evidence from other regions being later (Hope and Golson 1995: 825-826). Linguistics has so far revealed almost nothing about the technology of early TNG speakers.

The structural and lexical diversity of TNG languages spoken along the central cordillera of the island is consistent with a dispersal across this region at least 5,000 years ago and possibly earlier. Although the diversity within some of the putative major subgroups such as Madang suggests an origin dating to at least 4,000 years, certain lowland groups in southwestern New Guinea (especially the Asmat-Kamoro) appear to be internally homogeneous, suggesting a relatively recent expansion of related languages from central and southern New Guinea into land that was below sea level until the last couple of thousand years (Pawley n.d.: 36).

The greatest concentration of other non-Austronesian phyla and isolates is found on the north side of the central mountain range from the Bird's Head to the Sepik-Ramu Basin. However, these are not well studied (Foley 1986). The three largest are the putative Sepik-Ramu phylum of about 100 languages spoken along the Sepik River and in the western part of Madang Province; the Torricelli phylum of about 48 languages spoken in the Torricelli Ranges; and the West Papuan phylum comprising about 24 languages spoken at the west end of the mainland, on the northern part of the Bird's Head and on North Halmahera Island. A number of smaller phyla are identified in the north of New Guinea, including Sko, Kwomtari, Amlo-Musiai and Left May.

Finally, there is the question of whether there is a remote relationship between non-Austronesian languages and Australian languages spoken by contemporary Australian Aborigines. Early attempts to link modern

Australian Aboriginal languages with non-Austronesian languages used linguistic typological and ethnological evidence, such as the appearance of similar weapons in some Sepik populations to those found in some Australian Aboriginal groups (Wurm 1983). However, ethnological evidence of this kind is irrelevant to the question. More recently some typological evidence of deep genetic roots between non-Austronesian and Australian languages has been proposed (Nichols 1997).

1.6 Conclusion

Archaeology and linguistics provide an important base for the investigation of the origins and prehistory of populations. Archaeology can provide information such as how long people have been in a region, details of their culture and environmental impact over a broad timescale. In contrast, historical linguistics provides information about language development over a period of about five thousand years. It is clear that the islands of New Guinea and Near Oceania have been populated for a very long period of time, allowing much opportunity for diversification. However, neither archaeology or linguistics have much to say about the relationship of the founding populations of the tropical north to the ancestors of the Australian Aborigines. They are equally silent on the question of subsequent immigration or external influence until the appearance of the Austronesians in the Bismarck Archigelago about 3,500 BP in the guise of the Lapita culture.

Whilst there is evidence of Pleistocene activities in the Bismarck Archipelago and the central highlands of the mainland, the record is much more limited for the mainland lowlands and blank for the highlands fringe. The situation almost everywhere is worse up to the early to middle Holocene. Yet on the mainland this seems to be the period of the genesis

and spread of the TNG phylum and of the development of agriculture, according to one reading of the evidence, in the upper Wahgi and Baliem. We have no indications about the affiliations of the TNG languages, nor is it known whether early/mid-Holocene agriculture, if it existed, was an independent development based on New Guinea plants or was brought in from outside with introduced plants or occupied some position in between. It was also in the early Holocene that rising sea levels separated New Guinea from Australia at the Torres Strait (Chappell 1976).

Much more is known about the Austronesian speaking people who arrived in the Bismarck Archipelago about 3,500 BP bringing the Lapita culture, a fully developed agriculture based on root and tree crops and equipped with domesticated animals and a rich material culture that included a number of innovations among which pottery making is likely to be one. The immigrants were responsible for the first settlement of the islands of Remote Oceanic beyond the end of the Solomon chain and this seems to have occurred before 3,000 BP, soon after their arrival in the Bismarcks. Some of the newcomers, however, settled in the islands of Near Oceania, where they entered into relationships with long-resident communities. The result was a complex process of integration whereby the immigrant culture was to varying extents indigenised. This is what gives island Melanesia its character and its complexity, a complexity increased by later settlement and/ influence from Polynesia and Micronesia. A parallel process of similar complexity took place with Austronesian settlement along the mainland New Guinea coast.

Chapter 2: The Peopling of New Guinea in the Context of Melanesia: human biological evidence

2.1 Introduction

The origins and interrelationships of New Guinea populations have been the centre of speculation by physical anthropologists for at least two centuries. Early hypotheses were based on gross morphological characteristics which grouped them with a variety of dark skinned peoples including negroes. However, as techniques for genetic, skeletal and statistical analysis developed, a closer relationship between these populations and contemporary regional populations became apparent.

Whilst archaeology and linguistics provide information about the origin, subsequent prehistory and interrelationships of New Guinea populations, many questions such as the number and nature of the successive waves of colonisation prior to the arrival of Austronesian-speaking populations in the region, remain unanswered. The development of modern techniques in biological anthropology provides a complimentary way of investigating these issues. Genetic markers provide a high level of accuracy in tracing population origins and interrelationships as they are able to identify likely population movements and interrelationships that cannot be detected otherwise. This chapter reviews the genetic and skeletal evidence for the peopling of New Guinea including the distribution of red blood cell polymorphisms associated with malaria as malaria has been a major determinant of population settlement in New Guinea (Brookfield 1964, Riley 1983, Groube 1993).

2.2 Genetic Evidence

2.2.1 Unique allele distributions

Kirk (1980) identified three broad patterns of unique allele (blood groups, red cell enzymes and serum proteins) distributions relevant to the origins of New Guinea populations. The first of these is represented by the distributions of the transferrin allele Tf*D1 and the GC*1A1 allele of the vitamin D binding protein system. Found in Australian Aborigines, nearly all New Guinea populations and throughout much of the rest of Melanesia, their distribution supports the hypothesis that these populations once shared a common gene pool.

The second pattern is a group of alleles including phosphoglucomutase (PGM)1*3, PGM2*9, PGM2*10, phosphoglycerate kinase (PGK)*4 and malate dehydrogenase (MDH)*3 detected at high frequencies in the New Guinea highlands and at lower frequencies in the lowlands and parts of Near Oceania. These alleles are commonly detected in Southeast Asians but not in Australian Aborigines suggesting these variants were newly introduced after the formation of the Torres Strait. PGM1*3 has also been detected in the West Carolines and Fiji.

The third pattern is 'Austronesian' and comprises a group of alleles including PGM1*7, PGK*2, Albumin *NG, thyroxine-binding globulin (TBG)*S, glutamic pyruvic transaminase (GPT)*3 and GPT*6 detected at highest frequency in Near Oceania, the north and east coast of New Guinea, the West Carolines and Fiji (Kirk 1980, 1992). Australian Aborigines do not have any of these alleles and they are rare in New Guinea highland populations. I have not included Haemoglobin (Hb)*Tongariki in this group as it probably originated in northern Vanuatu before spreading northwest as the result of backmigration (Higgs et al. 1984).

2.2.2 Identification of a unique Austronesian marker

Although there are exceptions, the linguistic dichotomy between Austronesian and non-Austronesian-speaking populations generally provides an obvious means of making inferences about population histories. However, attempts to identify genetic markers uniquely associated with Austronesian-speakers have remained inconclusive (Serjeantson et al. 1992). The best known of these is a study of the gamma gobulin (Gm) system in Markham Valley populations that reported significant differences in some alleles between people from Austronesian and non-Austronesian-speaking populations (Giles et al. 1965). For example, Gm^1 had a frequency of ten percent in Austronesian speakers and 26 percent in non-Austronesian speakers. They concluded that these differences were due to the separate biological origins of these populations and that Austronesian speakers are genetically closer to modern Southeast Asian populations. However, this relationship was unable to be confirmed in Solomon Island populations (Steinberg et al. 1972).

Reanalysis of the available Gm data from New Guinea and the Solomon Islands did not find any differences between Austronesian and non-Austronesian speakers apart from in the Markham River Valley (Terrell and Fagan 1975). However, principal components analysis of the data found three main population clusters comprising highlanders, Markham Valley populations and a cluster derived from Near Oceania that included both language groups (Rhoads and Friedlaender 1987). They concluded that Austronesian speakers from the Markham Valley and Near Oceania were intermediate between non-Austronesian speakers from the mainland and Near Oceania.

The original Markham River Valley study (Giles et al. 1965) had not taken into account the effect of altitude or the relatively recent arrival of highland derived non-Austronesian populations to the valley. A multivariate analysis of blood genetic markers, anthropometrics and dermatoglyphics for three groups of Markham River Valley villages attempted to correct for these factors (Froehlich

and Giles 1981). The three groups comprised a lowland Austronesian-speaking population and two groups of higher altitude non-Austronesian-speakers including an Anga population. The study showed that their biological and linguistic differentiation was almost certainly related to their past evolution in isolation from each other despite their present geographic proximity (Froehlich and Giles 1981).

Multivariate analysis using twenty-two polymorphic markers was able to distinguish between Austronesian and non-Austronesian-speakers on Karkar Island (Boyce et al. 1978). However it was unable to distinguish whether this was due to the effects of language or geography because the study populations were from different regions of the island. A later study of north coast populations that took geography into account was unable to differentiate between Austronesian and non-Austronesian populations in a range of north coast New Guinea populations on the basis of seventeen polymorphic systems (Serjeantson et al. 1983). A more detailed analysis was carried out on nine language groups in Bogia to analyse the relative effects of geographic proximity and language. It was concluded that geographic location was more important than linguistic differentiation. Reanalysis of the data using maximum likelihood methods supported this conclusion (Bhatia et al. 1995a).

2.2.3 Polymorphic systems

Data for a wide range of polymorphic genetic systems have been analysed by multivariate analysis. Early studies based on ABO and MNS blood group gene frequencies clustered New Guinea populations into four groups. These are Madang and Sepik provinces, highlanders, non-Austronesian speakers from New Britain and the Papuan south coast and Austronesian speakers from the New Guinea islands and the Papuan south coast (Booth and Simmons 1972).

Possibly the best known study of polymorphic systems in New Guinea is an extension of Keats' (1977) multivariate analysis of fifteen non-Austronesian-speaking and five Austronesian-speaking populations (Kirk 1982). In this study

Austronesian-speaking populations clearly separated from non-Austronesian-speaking populations, except for the Mailu, a non-Austronesian-speaking population surrounded by Austronesian-speakers with whom they share many cultural traits.

Multivariate analysis of 143 New Guinea and other western Pacific populations based on the ABO, MNS and Rh blood group systems and Gm markers (Rhoads 1983) showed general similarities between Austronesian-speaking mainland populations and island Melanesians. Highland populations clustered separately, although some Sepik and Markham Valley populations showed a close relationship to them. The analysis was able to discriminate between Austronesian and non-Austronesian-speaking populations fairly accurately. All misclassified Austronesian populations were from the periphery of the Markham River valley comprising former highland populations that had become Austronesian speakers but had not yet had time to differentiate genetically (Froehlich and Giles 1981).

We have seen how a population can speak an Austronesian language yet be genetically non-Austronesian. The reverse is also true. In the above study three populations were misclassified as non-Austronesian. These were the Mulia, a highly aberrant Irian Jaya population, as well as the Mailu and Koita which are both surrounded by Austronesian-speaking populations (Froehlich and Giles 1981). Other examples have also been found. For instance, the non-Austronesian-speaking Indonesian populations, including those from Halmahera, cluster genetically with Austronesian-speaking populations and are differentiated from non-Austronesian-speaking New Guinean populations (Sofro 1982).

2.2.4 Natural selection by malaria

Malaria has had a major impact on populations living in tropical regions because acute *falciparum* malaria is a leading cause of death in young children. Malaria is a particularly powerful selector because death occurs before reproductive age in less resistant individuals and several malaria-associated red blood cell variants that

confer protection against the various forms of malaria have been identified in exposed populations.

Malaria is endemic in lowland New Guinea, Southeast Asia and much of island Melanesia. Until recently it has been relatively rare in the New Guinea highlands and is not found in New Caledonia. Malaria has been an important determinant of settlement in New Guinea. Before European contact there were two relatively distinct altitudinal concentrations of people living there - those living at altitudes greater than 1300m and those living on the coast penetrating inland to an altitude of 600m (Parkinson 1974). Many malaria associated polymorphisms are found in the coastal and island regions of Melanesia. Their distribution provides information about past malarial selection in these populations.

2.2.4.1 Thalassaemia

The thalassaemias are genetic disorders of haemoglobin synthesis characterised by a reduced rate of production of one or more globin chains. There are many forms of thalassaemia, however, defects in either the α or β globin genes are the most clinically important. α thalassaemia is highly prevalent throughout Melanesia although both forms are found in New Guinea.

2.2.4.1.1 α-thalassaemia

α-Thalassaemia is found throughout Melanesia and Polynesia, including New Caledonia. It occurs in two forms both caused by the deletion of one or more α-globin genes. Normal individuals possess two α-globin genes per haploid genome located on the short arm of chromosome 16 (Deisseroth et al. 1977). The deletion of a single α-globin gene results in thalassaemia 2 (α^{+}-thalassaemia). Homozygotes for this condition have two α-globin genes (-α/-α) and heterozygotes have three α-globin genes (-α/αα). The homozygous form is characterised by mild hypochromic anaemia and there is usually no phenotypic expression of the heterozygous form. The deletion of a linked pair of α-globin

genes results in thalassaemia 1 (α^0-thalassaemia). Homozygotes for this condition (--/--) are stillborn whereas heterozygotes (--/$\alpha\alpha$) are phenotypically identical to thalassaemia 2 homozygotes and can only be distinguished from them using direct DNA techniques.

Most thalassaemia in Melanesia is the thalassaemia 2 form of the disease which is strongly correlated to malarial endemicity (Flint et al. 1986, Yenchitsomanus et al. 1986a) but the mechanism by which protection is moderated is not known (Serjeantson et al. 1992). Low frequencies of the heterozygous double deletion (--/$\alpha\alpha$) have also been reported and haemoglobin H (-α/--) occurs sporadically (Ryan et al. 1961, Booth 1966, Amato 1977). However, the frequency of the homozygous double deletion is so low that it has never been confirmed by molecular typing (Serjeantson et al. 1992). The distribution of the two forms of α -thalassaemia suggests that differential selection may be operating between them in New Guinea (Wills and Londo 1981).

There are four common single α globin deletion variants found in Melanesian and regional populations: a 4.2 kb deletion (-$\alpha^{4.2}$) and three 3.7 kb deletions (-$\alpha^{3.7}$I, II and III) each caused by a different crossover event. Their distribution has led to their use as genetic markers. In New Guinea all variants are rare or absent in highlanders (Flint et al. 1986). The -$\alpha^{4.2}$ deletion is most common along the north coast of New Guinea (Flint et al. 1986, Yenchitsomanus et al. 1986a) whereas the -$\alpha^{3.7}$I deletion is most common in Karimui (20 percent) and along the south-west coast of New Guinea (10-42 percent) (Yenchitsomanus et al. 1986b). The -$\alpha^{3.7}$II type is not found in Melanesia but is common in Australian Aborigines and Southeast Asians. In contrast, the -$\alpha^{3.7}$III subtype is common in island Melanesians and Polynesians and detected occasionally along the north coast of mainland New Guinea (Hill et al. 1985). A single triplicated α-globin chromosome, created as the reciprocal of the recombination producing the single deletion, is found at low frequencies in Madang, Port Moresby, Karimui and Goroka and at 8 percent in Wanigela (Yenchitsomanus et al. 1986a).

Central Australian Aborigines and New Guinea highlanders share some unique α-globin haplotypes suggesting a remote common origin that differs from modern Southeast Asians (Roberts-Thomson et al. 1996). The haplotype associations of the α-globin deletions found in Melanesia suggest they are likely to have originated in there (Flint et al. 1986). For instance, the $-\alpha^{3.7}$III subtype associates with a single restriction enzyme haplotype suggesting a single origin for the subtype probably in northern Vanuatu (Hill et al. 1985, Flint et al. 1986, Yenchitsomanus et al. 1986a). Its current distribution suggests that backmigration has occurred to the Bismarck Archipelago (Yenchitsomanus et al. 1986a). Micronesians have a low frequency of the most common Melanesian haplotype for all three deletions suggesting some gene flow has occurred between these populations (O'Shaughnessy et al. 1990).

2.2.4.1.2 β-thalassaemia

β-thalassaemia has a much greater clinical impact than α-thalassaemia. It is prevalent in the malarious lowland areas of New Guinea and occurs rarely in the highlands (Curtain et al. 1962, Giles et al. 1967, Hornabrook et al. 1972, Booth and Garo 1978, Vaterlaws et al. 1981). Most cases of β-thalassaemia are caused by point mutations of the β-globin gene (Hill and Wainscoat 1986) located on chromosome 11 (Deisseroth et al. 1978). Homozygotes require regular blood transfusions for survival whilst heterozygotes have varying degrees of morbidity.

In New Guinea β-thalassaemia is most prevalent along the east coast (4.7-31.5 percent) and is detected at much lower frequencies along the highly malarious north coast (1.5 percent) where the $-\alpha^{4.2}$ deletion occurs suggesting that preferential selection may be operating (Yenchitsomanus 1986a). Possibly the protective effect of β-thalassaemia is reduced by coinheritance of both defects because the reduced intraerythrocytic globin-chain imbalance produces less oxidative stress and fewer metabolic changes. Two haplotypes have been identified for β-thalassaemia in New Guinea, suggesting that it has originated at least twice on the island (Yenchitsomanus et al. 1986b).

Although early comparisons of β-globin haplotypes within New Guinea and with other regional populations were confusing (Chen et al. 1990), there is now strong evidence that the distribution of β-globin haplotypes align New Guinea highlanders with Australian Aborigines (Liu et al. 1997).

2.2.4.2 Glucose-6-phosphate dehydrogenase deficiency

Glucose-6-phosphate dehydrogenase (G6PD) is an X-linked enzyme that catalyses the first step of the pentose phosphate pathway. This reaction reduces nicotinamide adenine dinucleotide phosphate (NADP) to NADPH which acts as an electron donor in many biosynthetic pathways as well as regenerating reduced glutathione, a compound which helps protect cells against oxidative damage. G6PD is particularly important in erythrocytes because they lack other NADPH-producing enzymes.

G6PD deficiency is very common in many areas where malaria has been endemic. It confers a selective advantage against *falciparum* malaria in female heterozygotes because the intra-erythrocytic stages of infection are extremely sensitive to oxidant stress (Luzzatto and Mehta 1989). Most people with the deficiency are asymptomatic and only express the phenotype (haemolytic anaemia) when exposed to fava beans, certain drugs or infection, although there are some forms that cause haemolytic anaemia even in the absence of these agents (Vulliamy et al. 1992).

G6PD deficiency is found throughout most malarious areas of New Guinea with marked local and regional variations (Yenchitsomanus et al. 1986b). It is found at frequencies of up to nine percent in the Sepik, fifteen percent in Madang Province, fifty three percent in Morobe Province and ten percent in Central Province. G6PD deficiency is not found west of the Strickland Gorge but is found along the upper and middle Fly River at three to nine percent (Schuurkamp et al. 1989). The deficiency reaches up to seventeen percent in the islands off the north

coast of the mainland (Yenchitsomanus et al. 1986b) and has also been reported at eight to fourteen percent in the Solomon Islands (Ishii et al. 1984) and on Vanuatu at up to thirty nine percent depending on the island (Ganczakowski et al. 1995). It is not found in highlanders (Yenchitsomanus et al. 1986b).

Almost 400 G6PD variants have been described on the basis of agreed biochemical characteristics of which about eighty have been characterised at a molecular level (Vulliamy et al. 1993). In some cases the same mutations were found in variants which were biochemically different (Vulliamy et al. 1992). Of the twenty four variants described in New Guinea (Chockkalingam and Board 1980, Chockkalingam et al. 1982, Kirkman et al. 1968, Yoshida et al. 1973) only two have been characterised at a molecular level (Wagner et al. 1996). Both are found in Asian populations raising the possibility that they were introduced from Asia to New Guinea, although this has yet to be confirmed. Two G6PD variants have been detected in Solomon Islanders (Hirono et al. 1995). One of these is G6PD Union which has been reported in many populations throughout the world including Southeast Asians. Three variants including G6PD Union were identified in Vanuatu populations (Ganczakowski et al. 1995). Further molecular characterisation of G6PD variants will be necessary to evaluate the relationships between them.

2.2.4.3 Hereditary ovalocytosis

Hereditary ovalocytosis is common in coastal New Guinea populations. *In-vitro* (Kidson et al. 1981, Hadley et al. 1983), cross-sectional (Baer et al. 1976, Serjeantson et al. 1977), hospital-based (Babona and Amato 1976) and matched-pair (Cattani et al. 1987) studies have demonstrated that it has a protective effect against *vivax* and *malariae* malaria. This condition is found in frequencies of five to twenty percent in lowland New Guinea (Amato and Booth 1977) and at similar frequencies in several aboriginal south-east Asian populations (Ganesan et al. 1975, Sembiring et al. 1975, Lie-Injo 1976). It is absent in highlanders and rarely detected east of the mainland suggesting that it was probably introduced to New

Guinea before the arrival of the Austronesians (Serjeantson and Gao 1995). The advantages associated with possession of this mutation would have facilitated its spread throughout the coastal areas.

Hereditary ovalocytosis is caused by a nine amino acid deletion in the band 3 erythrocyte protein involved in anion transport (Jarolim et al. 1991, Tanner et al. 1991) resulting in decreased membrane deformability (Saul et al. 1984) so that most circulating erythrocytes are oval shaped with a length to width ratio greater than 1:1 but less than 2:1 (Amato and Booth 1977). There is also a tendency for some erythrocytes to have either a single or double slit of pallor (somocytes or knizocyte) and rouleaux do not form in the thicker regions of blood smears. It is sometimes confused with elliptocytosis, a different condition occasionally associated with chronic haemolysis as early descriptions of hereditary ovalocytosis in Southeast Asia were described as elliptocytosis before ovalocytosis was recognised as a separate condition (Prior and Pitney 1967).

Although rarely reported in highland populations (Amato and Booth 1977, Nurse 1980a), hereditary ovalocytosis is irregularly distributed across the centre of the island including high altitude regions where there is no parasitaemia (Holt et al. 1981, Schuurkamp et al. 1989). As this is the widest part of the island and the narrowest part of the central cordillera, it has been suggested that its presence in this region is either due to genetic exchange with lowland populations since the introduction of the sweet potato (probably about 300 years) (Schuurkamp et al. 1989) or represents the historical remnants of population movements southward from the north coast (Holt et al. 1981).

2.2.4.4 Gerbich bood group

Although most populations are universally positive for the Gerbich blood group, the negative phenotype is observed across the Torricelli foothills-Sepik swamp area, along the Markham Valley and north to the Garaina people (Booth et al. 1972) with a prevalence of about ten percent (Booth 1971). Epidemiological data

suggest that the negative phenotype may confer a selective advantage against malaria although the exact mechanism is not known (Serjeantson 1989a). The restricted distribution of this polymorphism suggests it was present in New Guinea prior to Austronesian settlement, possibly having arisen in one of the populations of the Torricelli Mountains where it is particularly prevalent (Serjeantson and Gao 1995).

Evidence for the suggestion that the negative phenotype may confer a selective advantage against malaria was provided by a study in the Gogol Valley near Madang which showed that the parasitaemia rate for *P. falciparum* and/or *P. vivax* infection observed in Gerbich negative individuals was 5.7 percent compared to 18.6 percent in Gerbich positive individuals (Serjeantson 1989a). However, no difference was observed for *P. malariae*. One phenotype in Melanesians is caused by a large deletion in the glycosporin C gene (Serjeantson et al. unpublished).

2.2.5 Population Histories - Female and Male Lineages

Female and male lineages can potentially be traced by analysis of mitochondrial DNA (mt DNA) and the Y chromosome. Although human mtDNA is an established genetic marker, technical difficulties have hampered the widespread use of the Y chromosome in population studies.

2.2.5.1 Mitochondrial DNA

Mt DNA is a valuable tool for population geneticists because of its matrilineal mode of transmission (Giles et al. 1980) and high mutation rate (Brown et al. 1979). There are at least 18 mtDNA lineages in New Guinea (Stoneking et al. 1990) and 15 mtDNA lineages in Australian Aborigines (Whittam et al. 1986). Both are derived from Asia but are unrelated to each other (Stoneking and Wilson 1989). Australian Aborigines are slightly less divergent from New Guinea

highlanders than coastal Melanesian, Samoan or Asian populations (Holst-Pellekaan S van 1997).

Mainland New Guineans show very little variation in mtDNA which is consistent with their small population size and geographic isolation (Stoneking and Wilson 1989). Coastal populations have less variation than highland populations (Stoneking 1986) and share many haplotypes with Malaysians (Ballinger et al. 1992). Whilst highlanders also have affinities with some Southeast Asian populations, they often lack other mutations associated with site losses or gains making clear associations difficult to interpret.

The deletion of one copy of a 9-base pair (bp) tandem repeat sequence (5' CCCCCTCTA 3') usually found in the non-coding region between the cytochrome oxidase II and the lysyl tRNA genes in human mtDNA has been widely used as an anthropological marker. Originally regarded as specific to Asian and Asian-derived populations (Wrischnik et al. 1987), it has since been found to occur independently in other populations including Africans (Viligant 1990, Chen et al. 1995, Soodyall et al. 1996), Europeans (Torroni et al. 1995) and Aboriginal Australians (Betty et al. 1996). However, Asian-derived populations with the deletion are likely to have descended from a single ancestral population (Ballinger et al. 1992) as they share the same haplotype characterised by T to C transitions at nucleotide positions 16,189 and 16,217 (Torroni et al. 1995).

The deletion is found in a clinal distribution from mainland Asia (3-18 percent) across the Pacific to Polynesia where is is found at near fixation (Hertzberg et al. 1989). It is found in coastal New Guineans and island Melanesians (8-12 percent) but not in highlanders or Australian Aborigines suggesting that it may be an Austronesian marker (Stoneking et al. 1990) although it is also found at near fixation in the Negritos and Aeta of the Philippines (Hertzberg et al. 1989). However, screening for the deletion in skeletal remains from a range of Lapita-associated sites only detected it in recent post-Lapita samples from Polynesia

(from 700 BP), the Central Pacific (300 BP) and Micronesia (200 BP) (Hagelberg and Clegg 1993). These results remain controversial because the earliest sites surveyed are of the late Lapita phase about a thousand years after Lapita first was introduced to the area.

A motif comprising three unique transitions at nucleotide positions 16,217, 16,247 and 16,261 in combination with the deletion has been identified that is almost completely restricted to east Indonesia, coastal New Guinea, the Bismarck Archipelago and Polynesia (Melton et al. 1995, Redd et al. 1995). It is thought to derive from a motif comprising the deletion with transitions at 16,217 and 16,261 (Redd et al. 1995) which can be traced through the Philippines to three Taiwan aboriginal groups (Melton et al. 1995). A single substitution at 16,217 is found throughout south-east Asia which is thought to have been spread extensively during early expansions. Two of the four Lapita samples with the 9-bp deletion had all three substitutions whilst the remainder had two (Hagelberg and Clegg 1993).

2.2.5.2 Y Chromosome

At this stage only a few studies have been carried out that include Melanesian populations (Breuil et al. 1987, Ellis et al. 1990, Mathias et al. 1994, Spurdle et al. 1994, Hammer et al. 1997). Y-chromosome DNA is much larger (approx. 60 Mb) and more complex than mtDNA. It has fewer known genes and contains many different sequence types including tandem and dispersed repeat families (Affara et al. 1994).

Although Y-chromosome polymorphisms were first reported over ten years ago, their use in tracing paternal lineages for population and evolutionary studies was hampered by difficulties in locating suitable polymorphisms (Jobling and Tyler-Smith 1995). Nevertheless, it is now possible to detect single nucleotide sites (Hammer 1994), microsatellite variation (Hammer et al. 1997) and long-range polymorphisms (Jobling 1994, Mathias et al. 1994).

Unfortunately most of the published studies that include Melanesian populations have too few samples to be meaningful or show little understanding of the differences between highland and lowland New Guinea populations or the relationship of Melanesians with other regional populations. The only wholly specific Melanesian study published to date showed that an Angan population, the Baruyas (n=21) were monomorphic for a haplotype so far not found in any other population (Breuil et al. 1987). This homogeneity may be the result of genetic drift caused by isolation over a long period of time (Spurdle et al. 1994).

2.2.6 Human Leucocyte Antigens

HLA is an effective tool in population analysis because it has a high degree of allelic polymorphism and each ethnic group has its own characteristic profile. HLA studies in New Guinea populations have consistently shown a restricted range of polymorphisms, particularly in highlanders (Serjeantson 1989b). Comparison with other regional populations suggests that highlanders and Australian Aborigines share a common ancestry whereas coastal groups have a greater affinity with island Melanesians than highlanders.

Principal component analysis of a wide range of New Guinea populations based on gene frequencies separated most of them into one of four clusters: highlanders west of the Strickland Gorge, highlanders east of the Strickland Gorge, populations from the Madang area and a cluster comprising Tolai speakers from New Britain, Boiken speakers from the Sepik, Mareng speakers who geographically cluster with the first group and Wahgi speakers who geographically cluster with the second group (Smith et al. 1994). However, reanalysis of the data using haplotypes showed they are correlated with both language and geography suggesting either multiple origins or diverse selective pressures operating on these populations (Bhatia et al. 1995b). HLA studies are reviewed in more detail in the second part of the thesis.

2.3 Skeletal Evidence

Early studies of human skeletal remains in Oceanic and regional populations were limited by the lack of material from non-Austronesian populations (Giles 1976, Howells 1976,1979, Pietrusewsky 1983). These studies clustered Tolai speakers with Tasmanian Aborigines (Giles 1976, Howells 1976). However, subsequent analysis showed that coastal New Guineans are distinct from Australian Aborigines, including Tasmanians, except for northern Australian Aborigines which were closely related to populations from the Gulf District (Pietrusewsky 1983). Later comparison of craniofacial variation in regional populations confirmed that Melanesians are biologically distinct from Australian Aborigines although they share ancestral ties with them (Pietrusewsky 1990). Modern Asian and Oceanic populations formed a separate cluster.

Within New Guinea, a marked separation was observed between Trobriand Islanders and the Markham River Valley, Gulf of Papua, Nebira and Eriama for metrical traits in cranial specimens (Pietrusewsky 1983). For non-metrical traits, a series from Biak Island, Irian Jaya, clustered with individuals from the Sepik and Purari Delta. Further analysis of cranial specimens including those from non-Austronesian populations confirmed that New Guinea is biologically heterogeneous (Green 1990: 368). The central highlands were essentially homogeneous with respect to craniometric variation suggesting that these populations have been isolated from coastal/lowland areas for a long time.

Comparison of fragmentary skeletal and dental material recovered from Lapita sites in the Mussau Islands dating at 3,600-2,500 BP with contemporary Indonesian and Melanesian material suggests that they have closer affinities to modern Indonesian than Melanesian populations (Kirch et al. 1989). This is consistent with an earlier study that compared Late Lapita (2,500 BP) skeletal material from Watom Island, off New Britain, with modern regional populations (Pietrusewsky 1975). Multivariate analysis clustered the Watom material with early material from Fiji and Tonga. The Lapita material was generally separated

from modern Oceanic populations, although there was a suggestion of a possible relationship to modern eastern Melanesians. The analysis placed Polynesians with Asians, well differentiated from Melanesian samples. Morphological features shared with modern Oceanic populations include rocker jaws, tall stature and costo-clavicular sulci. Differences include small teeth, slender lower long limb bones and short broad mandibles. Preliminary analysis of a recent Lapita find from Waya Island, Fiji, dating at about 2,700 BP, suggested affinities with Polynesians and Southeast Asians (Pietrusewsky et al. 1997).

2.4 Conclusion

Studies based on genetic markers and skeletal analysis have consistently supported the hypothesis that there is a remote relationship between Melanesians, particularly New Guinea highlanders, and Australian Aborigines (Kirk 1980, Pietrusewsky 1990, Serjeantson 1989b, Gao et al. 1992, Roberts-Thomson et al. 1996, Liu et al. 1997) although these populations are biologically distinct from each other. Although archaeology and linguistics are unable to distinguish between the various waves of migration into New Guinea prior to the arrival of the Austronesians, genetic analysis suggests that mainland New Guinea was colonised by at least two biologically distinct groups of people before the arrival of the Austronesians (Kirk 1980). The relationship of this population movement to the development of agriculture, the introduction of new varieties of flora and fauna, including possibly the pig, and the spread of the TNG phylum of languages is not known.

More is known about the arrival and spread of the Austronesians. However, no unique Austronesian markers have been identified as several Austronesian-speaking populations have been identified that are genetically non-Austronesian and vice versa (Froehlich and Giles 1981, Sofro 1982) making clear associations between genetic markers and language group difficult to interpret. Skeletal analysis of remains from Lapita associated sites, have consistently shown that early Austronesians were closer to modern Southeast Asians and Polynesians than

Melanesians (Kirch et al. 1989, Pietrusewsky et al. 1997). However, analysis of mtDNA from these sites suggests that there may have been more than one wave of people moving through Melanesia to colonise Polynesia (Hagelberg and Clegg 1993).

The distribution of red blood cell polymorphisms associated with malaria, such as ovalocytosis and the Gerbich negative blood group, suggests that malaria was present in Melanesia before the arrival of the Austronesians. Their present distribution in coastal regions suggests a complex interplay of protection between the different forms.

The likely origin of Hb*Tongariki and $-\alpha^{3.7}$ III globin deletion in northern Vanuatu and their present clinal distribution northward provides genetic evidence of backmigration (Flint et al. 1986). Genetic analysis provides evidence of gene flow between Melanesia and Micronesia (O'Shaughnessy et al. 1990).

Chapter 3: Human Leucocyte Antigens (HLA)

3.1 Introduction

The purpose of this study is to use the HLA class I profile for each of eight Melanesian populations to draw inferences about the peopling of New Guinea. HLA are cell surface glycoproteins which present foreign antigens to T-cells. There are two classes of HLA: class I antigens which include the classical class I antigens HLA-A, B and C and class II antigens comprising HLA-DR, DQ, and DP. These are useful in population studies because they are extremely polymorphic and show marked variation in allele frequency and linkage disequilibrium between populations. Although many disease associations with HLA have been reported, particularly between class II loci and autoimmune disease, the link between HLA and infectious disease is at best tenuous. This chapter reviews the structure and function of HLA proteins and genes, the generation and maintenance of HLA polymorphism and its distribution throughout Melanesia with particular regard to class I loci.

3.2 HLA Structure and Function

HLA class I antigens are expressed at the surface of many, but not all, nucleated cells, platelets and to a limited extent on reticulocytes (Rodey 1991: 12). They consist of a 44,000 dalton transmembrane heavy chain non-covalently associated with a 12,000 dalton light chain, β_2-microglobulin (β_2m) (Figure 3.1). The heavy chain is composed of three extracellular domains (α_1, α_2 and α_3), a transmembrane region and a cytoplasmic tail. Most polymorphism is located in the α_1 and α_2 domains (Orr et al. 1979) which influence the specificity of the peptide binding site located on them (Barber and Parham 1993). The rest of the heavy chain is more conserved and the light chain, β_2m, is monomorphic.

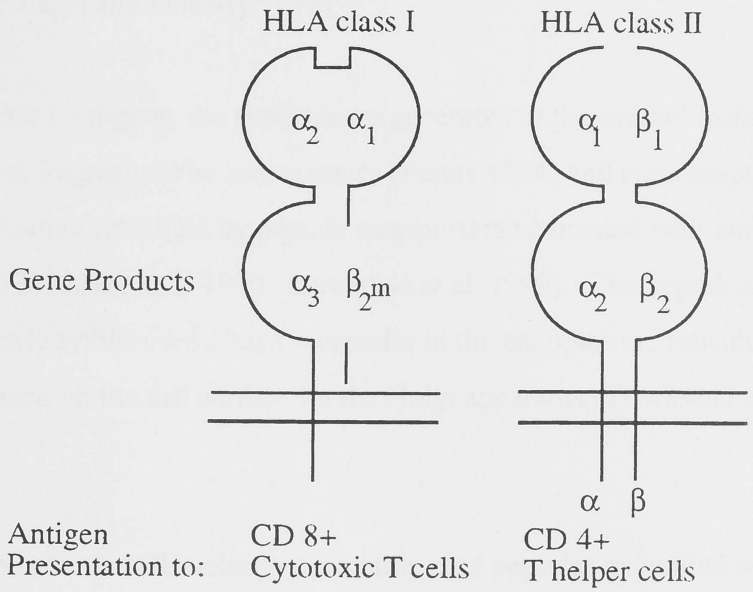


Figure 3.1: HLA class I and II antigens (de Vries and van Rood 1985)

In contrast, HLA class II antigens are expressed on macrophages and other antigen-presenting cells, B lymphocytes and activated T lymphocytes (Winchester and Kunkel 1979). They consist of two non-covalently associated transmembrane polypeptide chains, a 34,000 dalton α chain and a 29,000 dalton β chain (Kaufman and Strominger 1979). The extracellular part of each chain is folded into two domains with most polymorphism being expressed in the β_1 domain, although the α_1 domain is polymorphic on the DQ molecule. The α_3 domain of the class I heavy chain, the α_2 and β_2 domains of class II molecules and the constant domains of immunoglobulin have homologous sequences suggesting a common evolutionary origin (Cushley and Owen 1983, Hood et al 1983).

The function of HLA molecules is to stimulate immune responses by presenting antigenic peptides to T cells. Class I molecules mainly select small fragments (typically nine amino acids in length) of antigens of intracellular origin and present them to CD8+ cytotoxic T lymphocytes (Townsend and Bodmer 1989). They also affect the activities of natural killer cells (Storkus and Dawson 1991). Class II molecules select larger antigen fragments (20-24 amino acids) of extracellular origin and pass them to CD4+ T helper cells (Vignali 1994). MHC

polymorphisms determine the antigen specificity of T cells (MHC restriction) (Zinkernagel and Doherty 1974).

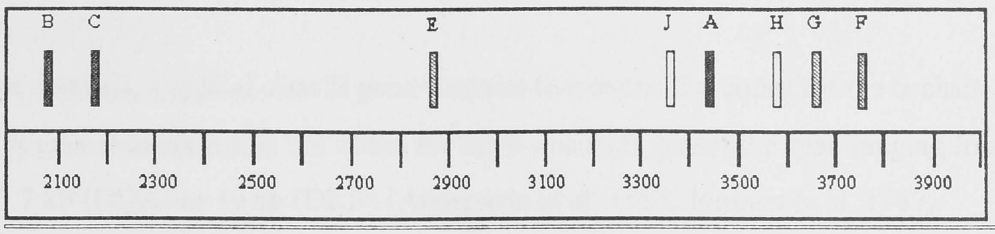
For class I antigens, the peptides are generated in the cytosol from denatured proteins fragmented by proteasomes (Peters 1994) and are transported into the endoplasmic reticulum by peptide transporters associated with antigen processing (TAP) (Monaco et al 1990, Trowsdale et al. 1990). The peptides associate with the newly synthesised class I molecules in the endoplasmic reticulum before being expressed on the cell surface via the Golgi apparatus (Townsend and Bodmer 1989).

In contrast, class II molecules primarily bind peptides generated by degradation of proteins in the endocytic pathway that internalises molecules from the cell surface via clathrin-coated vesicles and targets them to acidified lysosomes (Harding et al. 1991). Class II molecules associate with the class II-associated Ii peptide (CLIP) which blocks the binding site while directing them through the biosynthetic pathway to endosomal compartments where HLA-DM molecules remove the CLIP thus enabling antigenic peptide binding (Denzin and Cresswell 1995). The peptide-class II complex then moves to the cell surface.

3.3 HLA Genes

The genes encoding HLA are in the MHC located at the distal end of the short arm of chromosome 6 at 6p21.3 (Lamm and Olaisen 1985). This ~ 4 Mb segment of DNA comprises of three clusters of genes (Figure 3.2). The class I region of ~1.7 Mb is located at the telomeric end of the MHC and codes for the heavy chains of the classical transplantation antigens: HLA-A, B, and C. The associated light chain, β_2m, locus is located on chromosome 15 (Goodfellow et al. 1975). The class I region also codes for a number of non-classic class I antigens thought to be involved with leucocyte differentiation: HLA-E, F, G, H and X, as well as a number of pseudogenes (Bodmer et al. 1997a).

HLA Class I



HLA Class II

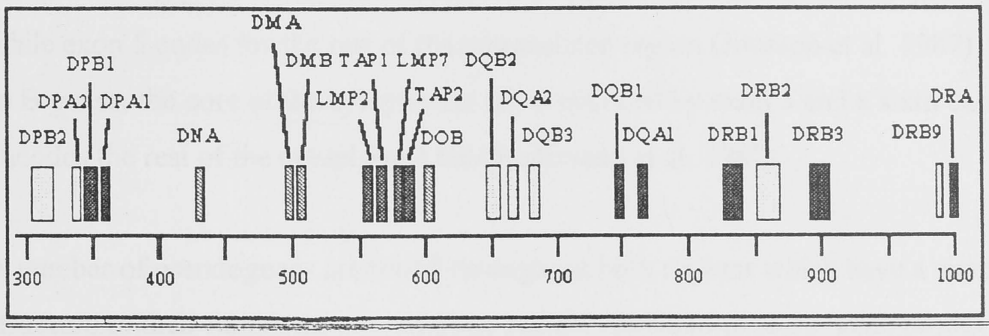


Figure 3.2: Overview of the major histocompatibility region on chromosome 6 (Marsh 1998)

The class II region of ~1.0 Mb is located at the centromeric end of the MHC comprising of at least 30 genes including HLA-DR, DQ and DP as well as a number of pseudogenes. The class III region of ~1.1 Mb is located between the class I and II regions and codes for a diverse selection of proteins including the serum complement components C2, C4 (C4A and C4B) and factor B; the microsomal enzyme steroid 21-hydroxylase (CYP 21) and cytokines TNF α and β

A typical HLA class I gene comprises seven or eight exons spanning 4-5 kb (Malissen et al. 1982, Gussow et al. 1987). The first exon (~ 75 bp) corresponds to the signal peptide (Cereb et al. 1995). Exons 2,3 and 4, (each ~ 270 bp) encode the first, second and third extracellular domains of the class I heavy chain respectively (Malissen et al. 1982). The fifth exon (~120 bp) codes for a short segment which is considered part of the third domain, the hydrophobic transmembrane segment and the beginning of the cytoplasmic region which anchors the antigen in the cell membrane. Exons 6 (~35 bp) and 7 (~40 bp) code

for the rest of the cytoplasmic region. Each of the exons are separated by noncoding introns of varying length.

In contrast, a typical class II gene contains five exons if it codes for the α chain (A genes) or six exons if it codes for the β-chain (B genes) the span ranging from 5.7 kb (DQA) to 19 kb (DRB) (Andersson et al. 1987, Jonsson et al. 1987, Gustafsson et al. 1987). These are arranged in a similar manner to class I genes except that there are only two extracellular domains. In A genes exon 4 codes for the connecting peptide, the transmembrane protein and the cytoplasmic segment while exon 5 codes for the rest of the untranslated region (Jonsson et al. 1987). In B genes, the core of the cytoplasmic tail is encoded by exon 5 and a sixth exon encodes the rest of the cytoplasmic tail (Andersson et al. 1987).

A number of pseudogenes are found throughout both regions which have a similar structure to their functional counterparts but are unable to produce gene products either because they have been truncated or because they have substitutions that disrupt the coding region (Geraghty et al. 1992). Limited polymorphism in class I pseudogenes has also been reported (Zemmour et al. 1990).

3.4 HLA Polymorphism: generation and maintenance

HLA is one of the most polymorphic functional genetic systems known. There are over 83 HLA-A, 186 HLA-B, 42 HLA-C alleles and 368 class II alleles identified to date (Bodmer et al. 1997a). The high degree of variability is thought to enhance the diversity of immune responsiveness. Most polymorphism in class I genes occurs in exons 2 and 3 which encode the first and second domains of the heavy chain comprising the peptide binding groove (Bjorkman et al. 1987) suggesting that natural selection has contributed to the polymorphism (Hughes and Nei 1988).

Many theories have been advanced to explain the high levels of HLA polymorphism observed including maternal-foetal incompatibility (Payne and Rolfs 1958, Rood van et al. 1959), disassortive mating (Potts et al. 1991) and unusually high rates of gene mutation (Bailey and Kohn 1965). However, there is little evidence to support any of these theories.

Today, it is generally agreed that a positive selection is responsible for MHC polymorphism. Of the various models proposed, the overdominant selection (heterozygous advantage) and frequency dependent models have been the most widely accepted. Overdominant selection assumes that a heterozygote should be able to present twice as many peptides and have twice the T-cell response as a homozygote thereby favouring the formation of sets of divergent peptide binding specificities through the accumulation of numbers of amino acid substitutions (Hughes and Nei 1988). This model is supported by the pattern of HLA-A polymorphism which comprise six distinctive allelic lineages. In contrast, frequency dependent selection assumes that an increase in the frequency of a new allele is favoured at the expense of older alleles until the organism adapts and it becomes an 'older' allele (Bodmer 1972). The strength of the selection is inversely correlated with the number of alleles in the population and the levels of heterozygosity. It is likely that some polymorphism is pathogen driven though direct evidence for a relationship between HLA and infectious disease is yet to be seen to ascertain the role of pathogens in generating and maintaining polymorphism. Each allele has a slightly different spectrum in terms of peptide binding and presentation, so a particular selective agent will have a different impact on each type of HLA molecule.

3.5 Molecular Evolution of Genes in the Class I Region

A histocompatibility system is found in all vertebrates. Comparisons of histocompatibility sequences in humans and higher primates show that many polymorphisms are shared by these species suggesting that they originated in a common ancestor (Lawlor et al. 1990a, Parham and Lawlor 1991). HLA

diversification is created by the accumulation of gene mutations over a long period of time, the main mutations being point mutation, segmental transfer and haplotype recombination. The existence of considerable locus specificity in the polymorphic regions of class I genes suggests that sequence exchange has not been a major contributor to their diversity (Parham et al. 1988). It is likely that class I antigens evolved from a class II-like progenitor more than 500 million years ago (Hughes and Nei 1993). It is thought that the progenitor for HLA-B and C diverged from HLA-A (Parham 1992) prior to gene duplication of HLA-B that resulted in HLA-C (Yeager and Hughes 1996).

The three class I loci differ from each other in a variety of ways. Phylogenetic and nucleotide sequence analyses of HLA-A alleles have shown that the alleles can be divided into five families (A2, A1/3/11, A9, A10 and A19) derived from two ancient lineages (Lawlor et al. 1991). A sixth family (A80) may be formed by the single allele A*8001, which has been found in African populations (Domena et al. 1993, Wagner et al. 1993). In contrast, HLA-B is the most diverse class I gene exhibiting greater sequence diversification in the peptide binding domains and sequence homogeneity in the α_3, transmembrane, and cytoplasmic domains than other loci suggesting that this locus may contribute more to immune function than the other loci. HLA-C has only ten percent surface expression compared to other HLA antigens (Snary et al. 1977). HLA-C also has fewer alleles than other class I loci which differ from each other by smaller numbers of substitutions, frequently at positions not directly involved in contacting the bound peptide and T cell receptor. This has lead to the suggestion that it may be a dispensible or declining locus (Parham et al. 1989, Lawlor et al. 1990b). However, the fact that HLA-C binds peptides, interacts with T cell receptors and is correlated with autoimmune disease suggests that it is not defunct but may have complementary functions to those of HLA-A and B (Zemmour and Parham 1992a).

3.6 Clinical and Anthropological Implications of HLA Typing

HLA typing has important implications for several fields of research including matching donors and recipients for transplantation; determining disease associations; and using HLA as a genetic marker in the study of the origins and relationships of different ethnic groups.

3.6.1 HLA matching and transplantation

Early studies of HLA centred on the fact that HLA polymorphisms elicit strong responses when tissues are transplanted between unmatched individuals (Klein 1975). Although early typing was based on serological techniques, molecular biology techniques based on nucleotide sequences are now routinely used in transplantation laboratories throughout the world. These techniques are able to detect serologically indistinguishable HLA subtypes, eliminate cross-reactivity problems and only require a small blood sample for each individual. The limitations of serological typing have consistently been seen in studies comparing serologically matched samples with DNA-typed samples which show frequent mismatching of HLA-A and -B subtypes (Madrigal et al. 1997).

3.6.2 HLA and disease associations

Many diseases, particularly autoimmune diseases, have been found to be associated with HLA polymorphisms. However although individuals with a disease may carry a particular HLA allele, only a small proportion of the total population with the allele develop the disease; the same disease may be associated with more than one allele; and, simple Mendelian segregation may not be observed. Estimation of the contribution of disease on selection for HLA is complex because the onset of HLA associated diseases is generally after the reproductive phase; environmental factors such as viral or bacterial infection may be involved; and, many HLA-associated diseases appear to be heterogeneous.

Disease	Associations	Racial Group	Relative Risk
Ankylosing spondylitis	B27	Caucasian/American Black/Oriental	54-69
Coeliac Disease	B8 DR3 DR7	Caucasian	7.6-11.6
Juvenile Diabetes	B8 B18 DR3 DR4	Caucasian/American Black	2.1-6.7
Juvenile Diabetes	B54 DR3 DR4	Oriental	2.6-5.6
SLE	B8, DR3	Caucasian	2.6-2.7
Tuberculosis	B35, DR 2	Chinese	Increased
AIDS	B35	Italian	Increased
AIDS	A1-B8-DR3	British	Increased
Malaria	B53, DR13.02	Gambia	Decreased

Table 3.1 Selected significant HLA disease associations (Lock and McDevitt 1992, Hill 1992, Hill 1996)

Most HLA-disease associations identified to date are non-infectious although infective agents may be involved in their induction (Hill 1992). The association of HLA with autoimmune disease is implicated by the fact that some polymorphisms have been shown to have a higher relative risk for certain diseases (Table 3.1). However, the mechanisms involved in HLA-associated autoimmunity are not well understood. One theory is that disease specific epitopes in the binding site of some HLA molecules may change some motifs critical to normal self peptide binding and conformation, triggering the immune system to attack self components (Todd et al. 1988). Alternatively peptides from some organisms such as the Epstein-Barr virus have homologous sequences to some HLA epitopes which could induce immune tolerance to the infectious organism or cause cross-reactivity by molecular mimicry (Roudier et al. 1989, 1991). Another possibility is that some pathogens such as adenoviruses develop mechanisms to block the presentation of their peptides by HLA molecules, thus avoiding the adaptive immune response (Peek et al. 1994). HLA-linked proteins such as complement may play an important role in some diseases such as systemic lupus erythematosus (SLE) (reviewed by Jonsson et al. 1995, Ruddy 1996).

Except for the progression of AIDS, an association between HLA and infectious diseases cannot be seen consistently across a range of studies. One difficulty

encountered has been the lack of consistent identification of alleles between different ethnic groups for a particular disease association. For example, tuberculosis is associated with HLA-DR2 in northern Indian populations (Rajalingam et al. 1996) but not in southern Indian populations (Sanjeevi et al. 1992). It has been suggested that an association between HLA and the progression of AIDS has been seen because it is a new disease and the host may not have had time to adapt by eliminating susceptible variants over a long period of time (Kaslow et al. 1996).

Malaria is likely to have acted selectively on HLA loci because it involves an immune response and death often occurs before reproductive age in less resistant individuals. A strong relationship between HLA and malaria has been reported from a large case-control study of children with severe malaria conducted in The Gambia (Hill et al. 1991). The study reported forty percent protection from both cerebral malaria and severe malarial anaemia from B53 and an association with severe malarial anaemia only from a class II haplotype, DRB1*1302-DQB*1*0501. However, a later case-control study conducted on the Kenyan coast found almost no difference between cases and controls, although a significant protective association was found with a different DR allele than that described from The Gambia (Hill et al. 1994). The difference between the two studies was attributed to different strains of *falciparum* in the two regions. No HLA association was found in children with mild malaria although the combined effects of all class II haplotypes indicated a significant association of morbidity with their distribution (Bennett et al. 1993).

Hill et al. (1992) used reverse immunogenetics to analyse the putative association between B53 and resistance to severe malaria. Crystallographic studies and sequence analysis of peptides eluted from B*5301 indicated a strong preference for proline as the anchor residue in the second position of the eluted self peptide, while there was no preferred anchor residue for the ninth position. Sixty peptides were identified and synthesised with proline at position two of their sequence from the primary sequence of the only four fully sequenced antigens known to be

expressed in the early stage of hepatocyte infection. These were assayed for binding to B*5301 using an HLA assembly assay (Elvin et al. 1991) Eight peptides were found to bind, including at least one from each antigen, one of these which was identified as a cytotoxic T lymphocyte epitope (Hill et al. 1992).

Although the putative alleles for malarial protection, B53 and DRB1*1302, are Africa-specific, there may be other alleles that provide protection against malaria in Papua New Guineans. However, a preliminary investigation, using serological techniques, failed to find an association between HLA and sporozoite and gametocyte epitopes in a population from Madang (Graves et al. 1989).

3.6.3 HLA and population studies

HLA is an effective tool in population studies because it is highly polymorphic and each ethnic group has its own characteristic HLA profile. Although some alleles are found in all racial groups, others are confined to a particular racial or ethnic group. HLA is subject to natural selection which may play a significant role in shaping the HLA polymorphisms of contemporary populations and should therefore be interpreted in the context of other available gene markers in population studies.

The tendency of alleles at closely linked loci to occur jointly in individuals more often than would be expected by chance (linkage disequilibrium) can be used to test hypotheses of population fusion and subdivision. Linkage disequilibrium can arise from selection, founder effects, or be created by population fusion even if there is no disequilibrium in either of the original fusing groups (Degos and Dausset 1974). In large randomly mating populations linkage disequilibrium decreases geometrically in the absence of selective forces. The recombination fraction (1-r) per generation is used as a measure of linkage. HLA-B and C are the most closely linked class I loci and have a recombination fraction of 0.2 per cent (Bodmer and Bodmer 1978) meaning that it would take 20,000 years of random mating to reduce linkage disequilibrium at this locus by a factor of five.

However, it would take HLA-A and B only 5,000 years of random mating to reduce linkage disequilibrium by a factor of five as they have a recombination fraction of 0.8 per cent (Robson and Lamm 1984). Deviation from random mating patterns can be calculated from heterozygosity data.

Studies made of the relationship between populations and ethnic groups using genetic distance values based on HLA frequencies have shown that HLA distributions are consistent with hypothesised relationships based on other anthropological data. Therefore HLA can be used to determine not only the origins and relationships of ethnic groups but also of subpopulations within these groups.

3.7 HLA in Melanesian Populations

3.7.1 Early serological studies

Attention turned to using HLA as a genetic marker for population studies when it became apparent that each ethnic group had its own profile (Bodmer and Bodmer 1970, Kissmeyer-Nielsen and Thorsby 1969, Ting and Morris 1972). At the time the first studies of HLA in New Guinea populations were published in the early 1970's serological techniques defined only a few HLA antigens at two loci: HLA-A and B. These studies revealed that mainland New Guineans, particularly highland populations, display a restricted range of alleles (Morris et al. 1971) although all alleles detected in these populations were also commonly detected in Asian populations (Ting and Morris 1972). Although greater allelic diversity was observed in coastal and Sepik populations than highlanders it was still more restricted than in Caucasian or Asian populations suggesting either that both populations shared a common ancestor with later admixture in coastal and Sepik areas (Ting et al. 1972) or that highland and coastal populations had different origins (Morris et al. 1972). Australian Aborigines displayed an identical range of alleles to New Guinea highlanders with two additional antigens HLA-A2 and HLA-A10 (Bashir et al. 1972, Cross et al. 1972).

The first study that attempted to correlate HLA with language in New Guinea was a comparison of the HLA profile of Austronesian (Takia) and non-Austronesian (Waskia) speakers on Karkar island (Morris et al. 1972). Both Karkar populations had a typical coastal profile suggesting that considerable admixture had occured between them in the past despite their different language profiles. This study forshadowed the later finding that the subtypes of alleles found in Caucasians and New Guineans differ, as the sera used gave quite different reaction patterns to those observed in Caucasian samples.

A meta-analysis of HLA distributions in sixteen Pacific populations collated from published and unpublished reports suggested that Australian Aborigines and New Guinea highlanders share a common ancestry and that New Guinea coastal groups show greater affinity with island Melanesians than with highlanders (Serjeantson 1982). There was a clear cut division between Melanesia and Polynesia.

The absence of HLA-A2 from New Guinea highlanders is of interest because A2 is found in all other world populations including Australian Aborigines and Oceanic populations (Serjeantson 1982). As no HLA-A2 was found in highlanders, the degree of Austronesian admixture in Melanesian populations was calculated based on the gene frequency of HLA-A2 in pooled Polynesian samples. The Austronesian admixture was estimated to be 15 per cent in coastal New Guinea, 16 per cent in Ouvea, 18 per cent in New Caledonia and 43 per cent in Fiji.

Only limited haplotype data were available for discussion. HLA-B13-Cw4 was found thoroughout Melanesia and in Australian Aborigines and HLA-A11-B40 was found in New Caledonians, Fijians and Australian Aborigines suggesting gene flow between these populations until at least thirty thousand years ago (Serjeantson 1982). In contrast, HLA-A9-B22, commonly found in Japanese but not detected in Melanesia, was restricted to West Polynesia and Fiji suggesting an Asian origin for these people with little gene flow into Melanesia.

3.7.2 Serological studies using redefined segregant series

In 1973 a third HLA locus (later designated HLA-C) was reported to lie between the two previously reported loci. (Svejgaard et al. 1973). As serological techniques developed, several antigen splits in the three loci were detected. The development of the mixed lymphocyte culture test (Bach and Hirschhorn 1964, Bach and Voynow 1966) led to the discovery that the mixed lymphocyte reaction was controlled by genes located on a separate locus officially designated HLA-D in 1975 (Thorsby and Piazza 1975). Later attempts to define the HLA-D locus by serology led to the establishment of the HLA-DR system (Bodmer et al. 1978). HLA-DQ and HLA-DP antigens were not established until 1984 (Shaw et al. 1980, Albert et al. 1984).

The redefined segregant series was used to reassess the HLA profiles for highland and coastal New Guinea populations (Bhatia et al. 1984, Crane et al. 1985). Two highland populations, the Asaro and Watut, were compared with each other and a group of unrelated coastal New Guineans temporarily resident in Sydney mainly derived from the north coast of New Guinea (Crane et al. 1985). The study found that highland populations are characterised by a low number of alleles at each locus, low average heterozygosity and number of haplotypes and extremely high antigen frequencies of HLA-B56, DR2 and DR14. The HLA polymorphisms of the coastals were less severely restricted than the highlanders being more similar to other Oceanic populations. The significant differences between the two populations suggested a separate origin from a similar stock (Crane et al. 1985).

These studies also provided new information about New Guinea population interrelationships. For instance HLA-A19 and HLA-A28 was not found in any study population (Bhatia et al. 1984, Crane et al. 1985) as previously reported (Ting et al. 1972). HLA-A11 was not detected in the Watut and only at low levels in the Asaro although it had a gene frequency of 25 per cent in the coastal group. Conversely, HLA-A24 was significantly less in the coastal group than

either highland population. The Watut was the only population with HLA-A23 and lacked HLA-DQ3. They also had the highest gene frequency of HLA-B56 ever reported. There was a high number of blanks for coastal individuals at the C locus and all population groups at the D locus.

Highland populations shared a number of HLA-B-C haplotypes with other Melanesian and Pacific populations including HLA-B13-Cw4 found in Australian Aborigines and island Melanesians and HLA-B60-Cw3 found in New Caledonia and Polynesians of the Wallis Islands possibly reflecting 10,000-15,000 years of isolation (Bhatia et al. 1984, Crane et al. 1985). In contrast, HLA-A34-B56 was observed in the Watut and coastal groups and HLA-B56-Cw1 and HLA-B56-DR1 in the Watut only (Crane et al. 1985). No significant linkage disequilibria were observed for HLA-A-B or HLA-B-DR in the Asaro (Bhatia et al. 1984).

3.7.3 Highland fringe populations

Examination of the HLA class I profile of highland fringe populations from the Karimui plateau and West Schrader Ranges (Haruai and Hagahai) provided valuable genetic information about their origins and affinities (Bhatia et al. 1988a, Bhatia et al. 1989). Karimui oral tradition suggests that they were originally riverine people from the south-west who have only recently made contact with highlanders (Nurse 1980, Russell et al. 1971). Although blood group data and cultural practices support this view (Russell et al. 1971), the HLA class I frequencies revealed significant admixture with nearby populations the Watut and the Daribi (Bhatia et al. 1988a). Two unique haplotypes were identified: HLA-A11-B56 and HLA-A24-B27. A11 associates nonrandomly with B27 in highlanders (Bhatia et al. 1988b). The Karimui have higher frequencies of HLA-A11 than highlanders, although it is lower than those recorded along the north coast (Morris et al. 1971, Ting et al. 1972, Morris et al. 1972 and Crane et al. 1985), and the highest frequency of HLA-B27 found in any world population (Bhatia et al. 1988a).

In contrast, there is no recorded oral tradition regarding the origins of the West Schrader populations (Haruai and Hagahai) although they are linguistically and culturally distinct from neighbouring highland groups (Jenkins et al. 1989). Significant differences in gene frequencies for HLA class I antigens were detected between these and other populations. Phylogenetic analysis based on these results showed that the West Schrader populations and Madang, the only coastal population included in the analysis, were well separated from each other and highland populations (Bhatia et al. 1989). The West Schrader populations had similar levels of HLA-A11 and HLA-A24 to northern coastal populations although both groups lacked HLA-A2 and HLA-B39 previously recorded in north coastal populations (Crane et al. 1985). However, they differed from each other in antigen frequencies and haplotype distribution (Bhatia et al. 1989). For example, the Haruai had the highest frequency of HLA-B62 recorded in New Guinea. HLA-B62-Cw4, a haplotype found widely across New Guinea (Bhatia et al. 1984, Crane et al. 1985) and HLA-B27-Cw1 a common highland haplotype, were found in the Haruai but not the Hagahai (Bhatia et al. 1989). The study concluded that the genetic profile of these West Schrader populations is the result of recent admixture between highland and coastal populations.

3.7.4 Melanesian population affinities based on serological data

A meta-analysis of twenty six populations comprising mainland and island Melanesians, Australian Aborigines, Polynesians and Micronesians was carried out based on published HLA data using the updated serological classifications (Serjeantson 1989b). The study concluded that Australian Aborigines and New Guinea highlanders share a common ancestry, as seen from HLA-B-C linkage relationships; that coastal New Guineans and island Melanesians have similar HLA distributions comprising a common base with Australian Aborigines and New Guinea highlanders with an Austronesian overlay; and that although early Austronesians left genetic influences in these areas they did not carry Melanesian elements into eastern Polynesia.

The analysis supported the hypothesis that there may have been more than one wave of people into the highlands (Bhatia et al. 1984) although the possibility of differentiation *in situ* after migration was not precluded. Evidence included high gene frequencies for HLA-A11 in West Schrader and Karimui populations compared to the low frequency observed among the Asaro and its complete absence in the Watut (Serjeantson 1989b). The analysis concluded that, based on restriction fragment length polymorphism (RFLP) analysis of HLA-DR antigens and the presence of a unique haplotype, HLA-A11-B56, West Schrader populations may have originated from an independent colonising event (Serjeantson 1989b) rather than coastal/highland admixture as previously suggested (Bhatia et al. 1989). HLA-A11 is associated with HLA-B40 in other Melanesian populations (Serjeantson 1989b) and HLA-B56 is usually associated with HLA-A34 in highland and coastal New Guineans (Crane et al. 1985).

The improved serological techniques enabled the more accurate analysis of HLA data for Melanesian populations. For example, HLA-A10, found throughout Oceania, splits into four subtypes A25, A26, A34 and A66 (Serjeantson 1989b). Of these, HLA-A34 occurs in Australian Aborigines, Melanesians and Polynesians whilst HLA-A26 is an Austronesian marker. Similarly, although HLA-B22 was detected in most Oceanic populations, its split into HLA-B55 and B56 showed that B56 is found exclusively in Australian Aborigines and New Guinea highlanders and is predominant in all other Melanesian populations whilst B55 is predominant in Polynesians (Serjeantson 1989b).

Similarly, typing of the C and D loci led to the definition of several previously unknown haplotypes linking the B and C loci, as well as the B and DR, a powerful tool in the analysis of the population interrelationships. Examples include: HLA-B13-Cw4 which is found in Australian Aborigines (Hay et al. 1986), New Guinea highlanders (Bhatia et al. 1984) and coastals (Crane et al. 1985) as well as New Caledonians but not other populations, suggesting a common origin for the haplotype and an independent source for Polynesians (Serjeantson 1989b). Similarly analysis of HLA-B-DR linkages showed B13-DR8 only occurs in

Australian Aborigines and New Guinea highlanders in contrast to B56-DR14, which is only found in New Guinea highland and coastal populations (Hay et al. 1986, Crane et al. 1985, Bhatia et al. 1984) implying there was more than one wave of migration to the highlands.

The meta-analysis included studies in which RFLPs were used to analyse class II alleles (Serjeantson et al. 1987, Jazwinska and Serjeantson 1988, Kohonen-Corish et al. 1988). RFLPs enabled finer discrimination of -DR alleles than serology. For example, unusual DR2 associated RFLPs were found in the West Schrader and coastal New Guinea populations using the DRβ probe (Serjeantson et al. 1987). This pattern is present in about 20 per cent of -DR2 positive Chinese and 10 per cent of -DR2 positive Japanese (Kohonen-Corish et al. 1988) but is absent in other populations.

Genetic distances were calculated using the presence or absence of DR-DQ combinations or HLA-A, B, C and DR data based on HLA data from the meta-analysis (Serjeantson 1989b). They confirmed that New Guinea highlanders were clearly differentiated from coastal and island Melanesians which clustered closer to Polynesians.

3.7.5 Broad population surveys

The most extensive survey of HLA class I associations in New Guinea to date was conducted across 49 populations mainly located along the central cordillera and its fringes but also including some north and south coastal populations, Manus Island and New Britain (Smith et al. 1994). The aim of the study was to determine the role of altitude and language in shaping the distribution of class I alleles in New Guinea using principal component analysis of class I data.

Considerable interpopulation variation was observed with populations clustering into one of four populations following principal coordinate analysis of antigen frequencies for the three class I loci. These were: Group A1 comprising several

highland populations located to the west of the Strickland Gorge and the Adzera, an Austronesian-speaking population from the Markham Valley; Group A2 comprising highland populations located to the east of the Strickland Gorge as well as the Bola, an Austronesian-speaking population from New Britain; Group A3 comprising of four groups from different locations - the Tolai from New Britain, the Boiken from the Middle Sepik, the Mareng who geographically cluster with the first group and the Wahgi who geographically cluster with the second; and, finally, Group A4 comprising of a mixture of Austronesian and non-Austronesian-speaking populations from the Madang area on the north coast. None of the six West Schrader populations included in the study clustered with these groups, although two of these populations, the Hagahai and Pinai, showed a closer affinity to Group A4 than the rest. Wanigela also remained unclustered although it was closest to the highland groups A1 and A2, and the Abelam, which include Wosera, were unrelated to any of the other study populations.

The study concluded that linguistic and altitudinal separation contribute significantly to the pattern of variation in HLA class I genes but that geographic distance was not a significant factor (Smith et al. 1994). Linguistic differences at the family or stock level within the TNG Phylum generally corresponded to genetic distances although the correlation was not observed within individual language families possibly due to intermarriage between groups or genetic isolation within a language group. In contrast, Austronesian speakers did not form a distinct cluster.

Populations were analysed to determine whether altitudinal differences could account for the variation in gene frequencies. Populations were classified into one of four zones: 0-600m, 600-1,200 m, 1,200-1,800 and above 1,800m. An altitudinal cline was observed particularly at the A locus. It was concluded that, based on heterozygosity data, the HLA distribution in New Guinea is partially under the control of selection operating differentially along the altitude gradient. Comparison of A locus heterozygosity between populations suggested that the

cline may be due to the absence of selection in favour of heterozygotes in the highlands rather than directional selection by any specific pathogens.

Reanalysis of the data using cluster analysis based on haplotype frequencies showed a correlation with both language and geography suggesting either multiple origins or diverse selective pressures on these populations (Bhatia et al. 1995b). Five clusters were formed: the first comprising lowland and island groups including the Hagahai, the second comprising eastern highlanders, Karimui populations and Abelem speakers, the third included groups from the Markham-Ramu valley and the Victor Emanuel Range near the West Irian border, the fourth comprised three Angan populations from the southern highland region and the fifth, comprised the several West Schrader populations including the Haruai. Wanigela was viewed as unclustered. Although the first two clusters are similar to A1 and A2 in the previous study there is no correlation with A3 or A4 in this study.

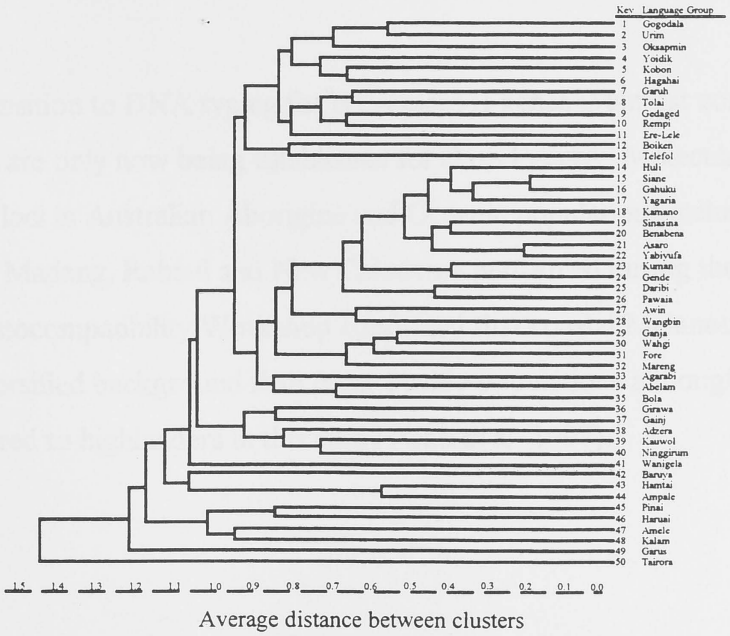


Figure 3.3: Dendrogram of New Guinea population relationships derived by the unweighted pair group method and based on three locus haplotype frequencies (Bhatia et al. 1995b).

3.7.6 DNA typing of class II HLA

The development of DNA typing has enabled the finest resolution of allelic variation including single base pair differences leading to the identification of many serologically undetectable subtypes. DNA typing of HLA class II alleles clustered New Guinea highlanders with Australian Aborigines rather than coastal Melanesians (Bhatia et al. 1992, Gao et al. 1992a, Gao and Serjeantson 1992a). In particular, highland populations lacked some alleles including DRB1*0403 and 1101 commonly found in coastal populations and had relatively high frequencies of DRB1*0410 and 1408 found in the same DR,DQ haplotypes in Australian Aborigines (Gao et al. 1992a, Gao et al. 1992b). Although coastal Melanesians shared some features, such as a high level of DRB1*1101 several regional differences were detected such as a negative cline in DRB1*1602 and 1104 from west to east. Finally, a number of novel DRB1 alleles were detected in Melanesian populations accounting for the HLA-D 'blanks' detected using serology.

Although the transition to DNA typing for HLA class II genes is almost complete these techniques are only now being established for class I genes. Molecular typing of class I loci in Australian Aborigine and Oceanic populations including individuals from Madang, Rabaul and New Caledonia performed during the 12th International Histocompatibility Workshop confirmed that coastal Melanesians have a more diversified background than other Pacific populations although they were not compared to highlanders in this study (Gao et al. 1997).

Chapter 4: Materials and Methods

4.1 Introduction

We used newly developed PCR/SSO based typing protocols to investigate the complete polymorphism of HLA class I genes in eight Melanesian populations to draw inferences about the peopling of Melanesia.

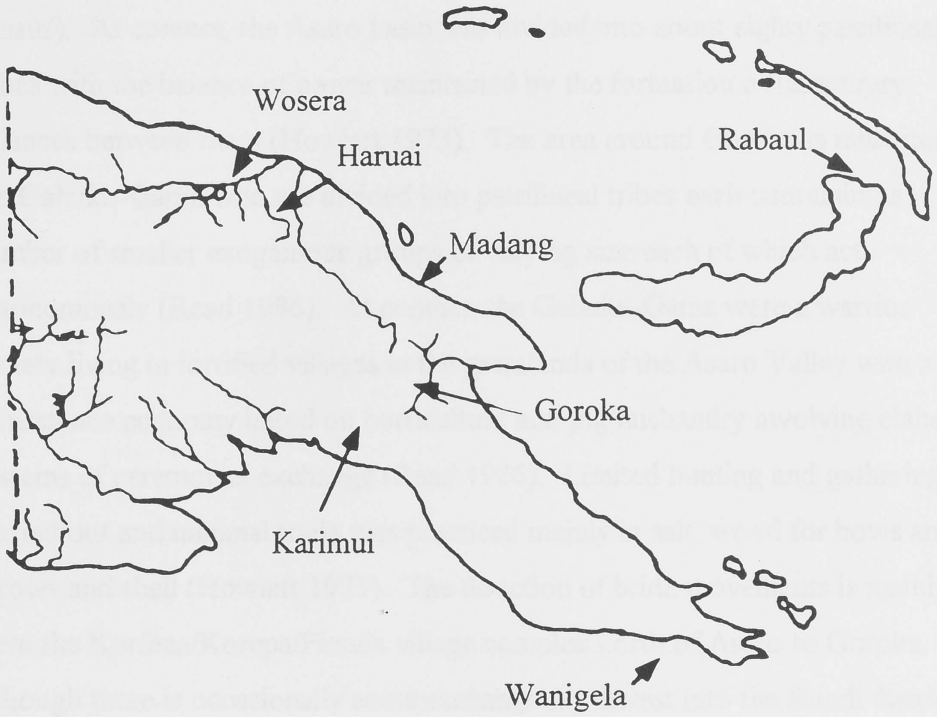
This chapter describes the details of the study populations and sample collection as well as the experimental methods used in this thesis. The experimental techniques had been previously developed in the Human Genetics Group, JCSMR (Gao et al. 1994). These protocols are able to discriminate the finest nucleotide sequence variations including single base-pair substitutions of known HLA class I alleles and to identify novel alleles in the study populations. The study was carried out as part of the ongoing research projects of the Human Genetics Group.

4.2 Study Populations

A total of 492 individuals were examined for HLA class I nucleotide polymorphisms. The samples were from eight Melanesian populations including highland, highland fringe and coastal New Guineans as well as samples from New Britain and New Caledonia.

The Papua New Guinea samples were collected by the Papua New Guinea Institute of Medical Research for on-going use in medical research. Consent was given by the donors prior to collection. Donors were from Goroka (n=35) in the East Highlands Province, the Haruai (n=55) in the northern highlands fringe, the Karimui Plateau on the southern highlands fringe (n=80), Wosera (n=131) in the Sepik-Ramu Basin, Madang township (n=55) on the north coast of Papua New Guinea, Wanigela (n=66) a coastal population from the southern tip of Papua and from Rabaul, on the Gazelle Peninsula of New Britain (n=28) (Map 4.1). The

Melanesian samples from New Caledonia (n=42) were the parents of families studied for HLA serology (Serjeantson et al. 1983) at the Second Asia-Oceania Histocompatibility Workshop in 1981.



Map 4.1 Papua New Guinea populations included in the study

Melanesian populations can also be classified linguistically. Non-Austronesian speakers include populations from Goroka, the Haruai, Wosera and the Karimui Plateau. Although the Madang, Wanigela and Rabaul populations all speak Austronesian languages they contain a strong non-Austronesian component. New Caledonia is generally considered to be purely Austronesian-speaking.

HLA typing of samples from Madang, New Britain and New Caledonia was carried out in the Human Genetics Group, JCSMR before the course of this thesis and the data were made available for the present analysis. HLA typing of the other populations was performed during the course of the thesis.

4.2.1 Goroka, eastern highlands

Goroka is located in the Asaro basin 1,600 metres above sea-level and was free of malaria at European contact. Like other highland areas, this region is densely populated with an average of thirty five people per square kilometre (1973 census). At contact, the Asaro basin was divided into about eighty patrilineal tribes with the balance of power maintained by the formation of temporary alliances between them (Howlett 1973). The area around Goroka is inhabited by the Gahuku-Gama who are divided into patrilineal tribes each containing a number of smaller exogamous groups of varying size each of which act autonomously (Read 1986). At contact, the Gahuka-Gama were a warrior society living in fortified villages in the grasslands of the Asaro Valley with a subsistence economy based on horticulture and pig husbandry involving elaborate systems of ceremonial exchange (Read 1986). Limited hunting and gathering was carried out and minimal trade was practiced mainly in salt, wood for bows and arrows and shell (Howlett 1973). The direction of bride movements is mainly from the Korfena/Korepa/Fionda village complex north of Asaro to Goroka although there is occasionally some exchange northwest into the Bundi district (Nurse 1981). Goroka lies on the Highlands Highway and is perceived by contemporary highlanders as 'towards the coast'. Gahuku-Gama and the other Asaro languages are members of the Gorokan family of languages which are related to the Kainantu family immediately to their east (Foley 1986). Both are members of the TNG phylum languages.

4.2.2 Haruai, northern highlands fringe

The Haruai are a group of about 700 individuals who live along the Arame River valley in the west Schrader ranges (Jenkins et al. 1989). Haruai languages form part of the Yuat super-stock of the Sepik-Ramu phylum together with Hagahai spoken by the populations immediately to their west (n=300), and Pinai located immediately south of the Hagahai along the Yuat River (n=250). Yuat populations are culturally distinct from surrounding populations that speak TNG

phylum languages (Jenkins 1987). Highland cultural traits include housing styles, clothing and body decoration, however, practices such as sister-exchange, betel-nut chewing and sago starch production are shared with lowland populations. In contrast, neighbouring TNG phylum populations are culturally highlanders.

The Haruai practice sedentary horticulture and pig husbandry in contrast to the Hagahai who are nomadic hunters and gatherers with limited pig husbandry (Bhatia et al. 1989). Although their territory ranges from 350-2,400 metres, few Haruai live below 1,100 metres thereby avoiding contact with malaria and filariasis. Over 85% of marriages are endogamous and monogamy is the rule with most marriages taking place within a parish of 16-100 individuals (Jenkins 1987, Jenkins et al. 1989). Consanguineal marriages with parallel and cross cousins are common.

It is not known how long the Haruai have occupied their territory although a Sepik origin has been suggested (Jenkins 1987). Indirect trade links have been documented reaching into the Western Highlands (stone axes), Enga (ash salt), the Kobon area (brine salt), the Sepik coast (shells) and the Gulf (pearl shell). There is archaeological evidence of extensive highland-lowland trade networks passing through the Pinai for more than 3,000 years, although these sites cannot be directly identified with the current inhabitants (Gorecki and Gillieson 1980).

4.2.3 Wosera, Sepik

The Wosera are a subgroup of Abelam-speakers of the Ndu family of Sepik-Ramu languages located on the foothills of the Prince Alexander Mountains. The Ndu language family is not related to any other known languages of the Sepik area (Laycock 1965). Populations speaking Ndu languages are ethnologically different to other Sepik groups (Thurnwald 1917, Kaufmann 1996). Within this family, Abelam is thought to be derived from the Iatmul located immediately south along the middle of the Sepik River. It has been estimated that they moved north from this area between a few hundred to a thousand years ago reducing the forest to

grassland by population pressure (Forge 1990, Swadling 1990). See Laycock (1965: 193) for a discussion of the probable origin of these populations. Before European contact, the Wosera were known to be warlike and land hungry (Lea 1973: 66). Today Wosera is the most densely populated lowland area in New Guinea (Lea 1973: 63) and has high malarial endemicity and morbidity, a large and accessible population and poor general health (Genton et al. 1995).

4.2.4 Karimui Plateau, southern highland fringe

The samples used in this study were collected from Pawaia speakers living in villages in the Sena River district of the Karimui Plateau. The Pawaia language is considered to be distantly related to Daribi and Polopa which together form a language isolate - the Teberan family (MacDonald 1973). Pawaia speakers are scattered over the southern highland fringe from the Karimui Plateau at 1100m down to much lower riverine environments such as Wabo at about 200 m. Although Pawaia speaking men commonly take wives from Daribi speakers located immediately to their west, Pawaia brides who leave the area marry downstream towards the coast (Nurse 1981). At the time of contact (1930-36), Pawaian and Daribi speakers located on the Karimui Plateau had an unusually high prevalence of leprosy approaching 8 per cent compared to 2.5 per cent in the rest of the Gulf Province (Nurse 1980b).

According to oral tradition they came from a coastal region further west, moving along the Erave Valley to the Upper Purari before spreading in several directions until they came in contact with the Daribi to the north, the Anga to the east and the Elema to the south (Warrillow 1978). At this stage they met resistance and migrated north. Linguistic evidence supports this model (MacDonald 1973). Blood group data and cultural traditions link them to coastal groups along the Papuan Gulf to the mouth of the Fly River (Russell et al. 1971). Based on linguistics the split between the Pawaia and the other Karimui populations is likely to have been quite ancient (MacDonald 1973). Archaeological evidence is lacking in the region as no major archaeological studies have been undertaken in the area

(Hide 1984: 19) because of the nature of the terrain (Nurse 1980b). Today the Karimui are agriculturalists (Bhatia et al. 1988) who live in communal rather than family groups (Shields et al. 1987). Polygamy is common so there is high proportion of half-siblings among samples.

4.2.5 Madang, north coast mainland

Madang is a moderate size town built on a peninsular midway along the north coast of New Guinea. There are small pockets of Austronesian are spoken along the coastline in the Madang district and on the nearby offshore islands. Austronesians along the north coast all have similar stories of population movement to the mainland from a large volcanic island called Yomba before it erupted and sank into the ocean ten to twelve generations ago (Mennis 1982: 3). Yomba Island is said to have been located at Hankow Reef.

Austronesians in the Madang area traditionally were a maritime culture which relied on trading of ceramics inland and across the Vitiaz Strait (Mennis 1982: 6, Harding 1967). Pottery ancestral to the modern industry has been found in many sites along the Madang coast dating from 550 BP (Egloff 1975). Trade and the close proximity of non-Austronesian-speaking populations have strongly influenced the Austronesian languages spoken in the area so that, for example, Gedaged has many typically non-Austronesian structural features (Foley 1986: 26).

4.2.6 Wanigela, southern Papuan tip

Wanigela village is situated on the Marshall Lagoon, approximately 200 km east of Port Moresby. All houses are built on stilts over the water ten minutes by motor boat from the Government Station at Kupiano. The Wanigela people established an urban squatter settlement in Port Morseby, known as Koki, in the 1950's. A house-to-house census conducted in Wanigela in 1983 showed that although the village comprised 306 houses, only two thirds were occupied as the

remaining population were temporarily residing in Koki (Patel et al. 1986). The female to male ratio exceeded 1.75:1.0. Wanigela people are one of the most susceptible populations to diabetes in the world (Dowse et al. 1994).

There is general agreement that Austronesian-speakers have been in Central Papua since about 2,100 BP (Vanderwal 1973, Allen 1977a, 1977b, Bulmer 1982). Papuan red-slip pottery appears in the area from 1,800 BP. Rapid cultural change occurred in this area about 1,000 BP which has been linked to the arrival of a second group of Austronesian-speakers from the east about this time which is detected archaeologically (Allen 1977a, Bulmer 1982) but not linguistically (Ross 1988: 195).

4.2.7 Rabaul, Gazelle Peninsula, New Britain

Rabaul is inhabited by Tolai speakers who live in a small area of the Gazelle Peninsula of New Britain which was relatively isolated from the rest of New Britain by the Baining mountains and dangerous seas until European contact (Epstein 1969: 8). At this time the only links with other communities were with the adjacent Duke of York Islands and the southern parts of New Ireland, although there were limited trading expeditions to Nakanai on the north coast of New Britain to acquire the *Nassa* shells which, when treated, were used as currency. The Tolai are the descendants of a population which migrated from the south of New Ireland following a massive volcanic eruption on the Gazelle Peninsula around 1,200 BP (Almond 1981, Walker et al. 1981) which destroyed the existing population (Specht 1968). This is supported by oral tradition (Salisbury 1972).

The Tolai speak an Austronesian language, Kuanua, which is closely related to those found on southern New Ireland (Ross 1988), although there has also been some borrowing from Nakanai as a result of trading (Chowning 1969: ~~before~~ 24). Kuanua bears little lexical resemblance to any other language spoken on New Britain and is surrounded by Baining, a non-Austronesian language spoken

immediately south of the Gazelle Peninsula (Chowning 1969 24). The population has expanded rapidly since European contact and today there are about 100,000 Tolai of which eighty per cent are located in rural villages of between 300 and 3,000 people surviving as cash crop farmers (cocoa and copra), subsistence farmers or artisans (Simet 1991). The Tolai are divided into matrilineal clans.

4.2.8 New Caledonia

New Caledonia comprises a large group of islands with a total land area of 19.1 km^2 located 1700 north of New Zealand. Its main island, Grande Terre, is 400 km long and 50 km wide. The Loyalty Islands (Ouvea, Mare, Lifou and Tiga) lie to the east of Grande Terre, to the north the Belap Islands and to the south the Isle of Pines. Grand Terre is divided by a mineral rich mountain range running north to south with peaks of up to 1600 metres. New Caledonia has been inhabited from about 3,200 BP and the earliest sites are associated with Lapita-ware (Spriggs 1997). Linguistic evidence suggests that it was originally peopled from the southern islands of Vanuatu (Geraghty 1989. Lynch 1996). There are twenty seven Austronesian languages spoken in New Caledonia today.

New Caledonia has a very diverse population comprising 43.3 percent Melanesians, 35.6 percent Europeans (mainly French), 12.6 percent Wallis Islanders and French Polynesians and 8.5 percent others mainly comprising Indonesians and Vietnamese. In 1983 there were an estimated 145,000 people living on the island (Kircher 1986). Before European contact there were an estimated 60,000 people living on New Caledonia in autonomous tribal communities along riverbeds, valleys and the foothills of the mountains. The coasts and mountains were only sparsely populated. The main island was discovered by Captain Cook in 1774. Interestingly his Journal records the presence of Tongan canoes along the east coast. France took possession of the main island in 1853 and used it as a penal colony from 1864 to 1904. It later became a rural settlement. Following European contact the indigenous populations were forced off their ancestral land to less fertile areas including the

interior. Their population dwindled from 42,500 in 1887 to 28,000 in 1901. Gold, chrome, cobalt and nickel were mined from the 1870's and today nickel mining is still the main economic activity. Most of the Wallis Island and French Polynesian population on the island work in the mining or building industries, whereas, most of the Asian population are employed in the agricultural and domestic sectors although some also own small businesses.

4.3 Experimental methods

4.3.1 DNA extraction

All DNA samples were prepared from buffy coats for previous studies using either pheno/chloroform extraction (Maniatis et al. 1982) or a salting-out procedure (Miller et al. 1988).

4.3.2 Primers

Previously designed locus-specific primers were used to selectively amplify exons 2 and 3 the most polymorphic region of class I genes which encode the peptide binding site (Table 4.1) (Gao et al. 1994 q.v. for other method details also). As the few sequence specific sites in exons 2 and 3 of HLA-A, B and C genes (Zemmour and Parham 1992b) occur mainly in the central region of each exon, primers were designed to selectively amplify the region from intron 1 to 3 comprising the entire exon 2-intron 2-exon 3. To achieve locus specificity for HLA-C amplification was carried out from the 5' region to include exon 1-intron 1- exon 2- intron 2-exon 3. PCR products were about 1090 bp for the A locus, 1040 bp for the B locus and 1080 bp for the C locus.

A second amplification using nested primers was carried out to optimise the reaction using PCR products from the first amplification as a template. The final product was about 950 bp at the A locus, 861 bps for the B locus and 846 bps for the C locus.

Primer	Sequence	Location	Specificity
First Amplification			
A1P5'	5' TCC CCA GAC GCC GAG GAT GGC C	5' UTR -16	A
A3N26P3'	5' CGG GAG ATC TAT AGG CGA TCA G	intron 3 +58	A
B1N60P5'	5' GGG AGG AGC AAG GGG ACC GCA G	intron 1 +38	B
B3N26P3'	5' CCC GGC GAC CTA TAG GAG ATG G	intron 3 +60	B
C5E36P5'	5' CCG GGT TCT TAA GTC CCC AGT C	5' UTR -58	C
BC3N1P3'	5' AAG GCT CCC CAC TGC CCC TGG TAC	intron 3 +24	C
Second Amplification			
A1N113P5'	5' GGT CGG GCA GGT CTC AGC CAC	intron 1 +92	A
ABC3NP3'	5' CCC GTG GCC CCT GGT ACC CG	intron 3 +17	A
BC2P5'	5' C CCC AGG CTC CCA CTC CAT G	intron 1+160	B
BC3N1P3'	5' AAG GCT CCC CAC TGC CCC TGG TAC	intron 3 +24	B
BC2P5'	5' C CCC AGG CTC CCA CTC CAT G	intron 1+160	C
C3P3'	5' CCC TGG TAC CCG CGC GCT GCA G	intron 3 + 9	C

Table 4.1: Primers used for nested amplification of HLA class I genes

4.3.3 PCR amplification

A nested PCR amplification was carried out at each locus. The first amplification was carried out in a 50 µl volume consisting of 0.2 µg genomic DNA; 15 pmoles of each primer; 0.2 mM of dATP, dCTP, dGTP and dTTP; 1 unit of Taq polymerase; 5 µl of 10X AB PCR buffer (200mM $(NH_4)_2SO_4$, 750 mM Tris-HCl pH9.0, 0.1% Tween 20 w/v); 1 mM $MgCl_2$; 5 µl of dimethylsulfoxide (DMSO); and an adequate volume of water. Thirty five amplification cycles were performed on a thermal cycler (Bartelt Instruments, Victoria) with each cycle consisting of 1 minute at 95°C, 1 minute at 59°C and 2 minutes at 72°C. The first cycle beginning with 3 minutes at 95°C and the last cycle ending with a 10 minute extension at 72°C.

The second amplification was performed in a 100 µl volume using 1.5 µl of the first PCR product as a template. The reaction mixture was doubled for amplification of the B locus. Each of the thirty five amplification cycles consist of 1 minute at 95°C, 1 minute at 56°C and 2 minutes at 72°C.

4.3.4 Dot blotting of PCR products

PCR products were mixed with an equal volume of 0.8M NaOH. 4 µl of PCR product mixture for each sample was dot blotted onto a nylon membrane (Amersham: Hybond N+) using an 8-channel pipette and then air dried. After dot blotting the membrane was baked at 60°C for 1 hour.

4.3.5 Oligonucleotide probes

The oligonucleotide probes, either 19 or 18 mer, had been previously designed on the basis of published HLA class I sequences and locally constructed on a 380B synthesizer (Applied Biosystems, Foster City, CA, USA). The number of probes used for HLA-A, B and C typing were 50, 100 and 50 respectively (Gao et al. 1994, Tables 4.2 and 4.3). Their hybridisation patterns are given in Appendix 1.

Oligonucleotide probes for use in hybridisation were end-labelled with ^{32}P by mixing 10 pmoles probe with 0.33 mCi of $\gamma^{32}P$- labelled adenosine 5'-triphosphate (4,000 Ci/mmole, Bresatec), 10 units of T4 polynucleotide kinase (Pharmacia, USA), 1.0 µl of 10x reaction buffer supplied with the T4 kinase, and water to a total volume of 10 µl. The labelling reaction was carried out a 37°C for 30 minutes.

4.3.6 SSO hybridisation

The blotted membrane was prewetted with water and prehybridised in a 50 ml plastic tube with 8 ml of a solution containing 15% deionised formamide, 10% of 50x Denhardt's solution, 5x saline sodium phosphate-EDTA (SSPE; 750 mM NaCl, 50 mM NaH_2PO_4, 5 mM Na_2EDTA pH 7.4), 1% sodium dodecyl suphate (SDS), 5% dextran sulfate, and 0.2 mg/ml boiled salmon sperm DNA. The prehybridisation was carried out at 42°C in a revolving hybridiser for 2 hours and 8 µl ^{32}P-labelled probe was then added into the solution. Hybridisation was continued for 1-2 hours at 42°C.

Table 4.2 Oligonucleotide probes for HLA-B typing

Name	Sequence (5′-3′)	Location	Name	Sequence (5′-3′)	Location
		Exon 2			Exon 3
B210	TTTCTACACCTCCGTGTCC	94-103	B304	CCAGAGGATGTTTGGCTGC	357-376
B211	TTTCTACACCGCCATGTCC	94-103	B305	CCCTCCAGTGGATGTATGG	353-372
B212	TTTCCACACCTCCGTGTCC	94-103	B306	CCCTCCAGAGGATGTACGG	353-372
B213	TTTCTACACCGCCGTGTCC	94-103	B307	CCCTCCAGAGCATGTACGG	353-372
B214	TTTCGACACCGCCATGTCC	94-103	B308	TCACACTTGGCAGAGGATG	345-364
B215	TTTCCACACCGCCATGTCC	94-103	B309	TCACACTTGGCAGACGATG	345-364
B220	CCGCTTCATCGCAGTGGGC	132-151	B310	TGCGACGTGGGGCCGGACG	373-392
B221	CCGCTTCATCACCGTGGGC	132-151	B312	TGTACGGCTGCGACCTGGG	365-384
B222	CCGCTTCATCTCAGTGGGC	132-151	B313	TGTATGGCTGCGACCTGGG	365-384
B223	CCGCTTCATTGCAGTGGGC	132-151	B314	ACCTGGGGCCCGACGGGCG	377-396
B230	GGACGACACGCAGTTCGTG	156-165	B315	TGCGACCTGGGGCCGGACG	373-392
B231	GGACGACACCCAGTTCGTG	156-165	B316	GCCCGACGGGCGCTTCCTC	384-403
B232	GGACGACACGCTGTTCGTG	156-165	B321c	GGCGGACTGGTCATGCCCG	405-414
B233	GGACGGCACCCAGTTCGTG	156-165	B322c	GGCGTCCTGGTGGTACCCG	405-414
B242	TCCGAGGATGGCGCCCCGG	198-217	B323c	GGCGTACTGGTTATGCCCG	405-414
B243	TCCGAGGACGGAGCCCCGG	198-217	B324c	GGCGAACTGGTTATACCCG	405-414
B244	TCCGAGAGAGGAGCCGCGG	198-217	B325c	GGCGAACTGGTTATGCCCG	405-414
B245	TCCGAGGAAGGAGCCGCGG	198-217	B326c	GGCGTACTGGTCATGCCCG	405-414
B246	AGCCCCGGGCGCCGTGGAT	209-228	B327c	GGCTAACTGGTTATGCCCG	405-414
B250	GCGCCGTGGATAGAGCAGG	217-236	B328c	GGCGGACTGGTCATACCCG	405-414
B251	GCGCCGTGGGTGGAGCAGG	217-236	B329c	GGCGAACTGGTCATGCCCG	405-414
B252	GGGGCCGGAGTATTGGGAC	237-256	B32A	CGGGTATGACCAGGAAGCC	405-414
B253	GGGGCCGGAGCATTGGGAC	237-256	B32B	CGGGTATAACCAGTTCGCC	405-414
B254	GGGGCCGGAATATTGGGAC	237-256	B32C	CGGGTATGACCAGTACGCC	405-414
B261	GAACACACAGATCTGCAAG	258-277	B331	CGGCAAAGATTACATCGCC	428-447
B262	GGAGACACAGATCTCCAAG	258-277	B340	CGAGGACCTGCGCTCCTGG	453-472
B263	GAACACACAGATCTCCAAG	258-277	B341	GGACCTGAGCTCCTGGACC	456-475
B264	GAACACACAGATCTTCAAG	258-277	B350	CCGCGGCGGACACGGCGGC	473-492
B265	GAACACACAGATCTACAAG	258-277	B355	CCCAGCTCAAGTGGGAGGC	493-512
B266	TTGGGACGGGGAGACACGG	249-268	B356	GGCTCAGATCTCCCAGCGC	489-508
B271	GCCTCCGCGCAGACTTACC	277-296	B360	GTGTGGCGGAGCAGCTGAG	524-543
B272	ATCTGCAAGGCCAAGGCAC	268-287	B361c	CTCAGCTGCTCCGCCTCAC	524-543
B273	CCAACACACAGACTGACCG	278-297	B362	GTGTGGCGGAGCAGCGGAG	524-543
B274	CAGATCTACAAGGCCCAGG	265-284	B363	GGCGGAGCAGTGGAGAGCC	528-547
B275	AAGTACAAGCGCCAGGCAC	268-287	B364	CGTGTGGCGGAGCAGGACA	523-542
B276	CCAACACACAGACTTACCG	278-297	B366	GCAGCTGAGAACCTACCTG	534-553
B281	GACTGACCGAGAGGACCTG	288-307	B370	GCACGTGCGTGGAGTGGTC	557-576
B282	GACTTACCGAGAGAGCCTG	288-307	B371	GAGGGCGAGTGCGTGGAGT	553-572
B283	GACTTACCGAGAGAACCTG	288-307	B372	GCCTGTGCGTGGCGTCGCT	557-576
B284	GACTGACCGAGAGAGCCTG	288-307	B373	GCACGTGCGTGGAGTCGCT	557-576
B285	GACTTACCGAGAGGACCTG	288-307	B374	GCCTGTGCGTGGAGTGGCT	557-576
B286	GCGGATCGCGCTCCGCTAC	306-325	B375	GCCTGTGCGTGGAGGGGCT	557-576
B288	GCGCACCGCGCTCCGCTAC	306-325	B376	GCCTGTGCGTGGACGGGCT	557-576
B289	GCGGAACCTGCGGGCTAC	306-325	B381	CGCAGATACCTGGAGAACG	577-596
		Exon 3	B382	GCTCCGCAGACACCTGGAG	573-592
B301	GTCTCACATCATCCAGGTG	342-361	B390	AGGAGACGCTGCAGCGCGC	599-618
B302	GTCTCACATCATCCAGAGG	342-361	B391	AGGACAAGCTGGAGCGCGC	599-618
B303	CCCTCCAGAATATGTATGG	353-372	B392	AGGACACGCTGGAGCGCGC	599-618

Probes marked c are complementary to the coding sequence

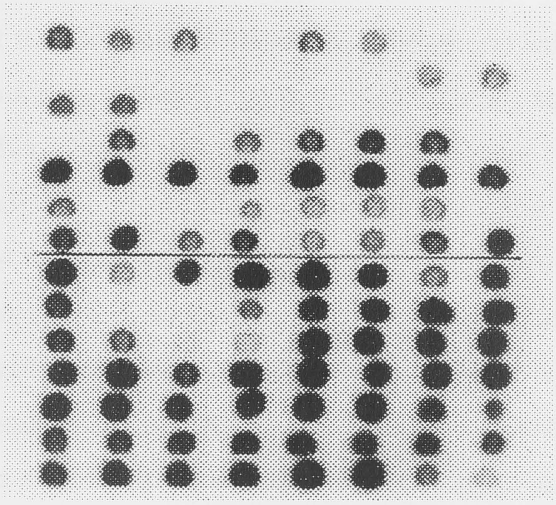
Table 4.3 Oligonucleotide probes for HLA-C typing

Name	Sequence (5'-3')	Location	Name	Sequence (5'-3')	Location
		Exon 2			Exon 3
C202	CACCGCTGTGTCCCGGCCC	98-117	C30A	CCAGTGGATGTTTGGCTGC	357-376
C203	CATCCGTGTCCTGGCCCGG	101-120	C312c	CGTCGGGCCCCACGTCGCA	363-382
C220	CGCGGGGAGCCCCGCTTCA	121-140	C313c	CGTCCGGCCCCAGGTCGCA	363-382
C222c	GAAGTGGGGCTCTCCGCGG	120-139	C321	CGGGTATGACCAGTCCGCC	405-424
C223c	GAAGTGGGGCTCCCCGCGG	120-139	C322	CGGGTATAACCAGTTCGCC	405-424
C231c	GTCGCTGTCGAACTGCACG	171-190	C324	CCTCCGCGGGCATGACCAG	309-328
C252c	CTCCACCCACGGCTCCCGC	213-232	C325	CGGGCATGACCAGTTCGCC	405-424
C254	GAGAGGGGAGCCCCGGCG	201-220	C32D	CGGGTACCACCAGGACGCC	405-424
C256c	CCGGGGCTCCCCTCTTGGA	198-117	C343c	GAGCCGCCTTGTCCGCGGC	475-494
C263	AAGCGCCAGGCACAGACTG	274-293	C344c	GTCCGCAGCGGTCCAGGAG	465-484
C264	AAGCGCCAGGCACAGGCTG	274-293	C345	CTCCTGGACTGCCGCGGAC	465-484
C271	GAGCCTGCGGAACCTGCGC	300-319	C351	GCGCAAGTTGGAGGCGGCC	504-523
C272	GAACCTGCGGAAACTGCGC	300-319	C352	GGCTCAGATCTCCCAGCGC	489-508
C273	GAACCTGCGGAAACTACGC	300-319	C361	GGCGGAGCAGCGGAGAGCC	528-547
C290	CCAGAGCGAGGCCGGTGAG	300-319	C362	GGCGGAGCAGTGGAGAGCC	528-547
C291	CCAGAGCGAGGCCAGTGAG	300-319	C363	GTGAGGCGGAGCAGCTGAG	524-543
		Exon 3	C365	CCGTGCGGCGGAGCAGCTG	522-541
C301	GGGTCTCACATCCTCCAGA	-1- 18	C366	GGCGGAGCAGGACAGAGCC	528-547
C302	GTCTCACATCATCCAGAGG	345-364	C367c	AGCTGCTCCGCCGTACGGG	521-540
C304	CCAGTGGATGTGTGGCTGC	357-376	C368c	CTCTCTGCTGCTCCGCCGC	526-545
C305	CCAGAGGATGTACGGCTGC	357-376	C371c	CTCCACGCACTCGCCCTCC	552-571
C306	CCAGAGGATGTATGGCTGC	357-376	C375	GCGTGGAGTGGCTGCGCAG	563-582
C307	CCAGAGGATGTTTGGCTGC	357-376	C376c	CAGGTATCCGCGGAGCCAC	570-589
C308	CCCTCCAGTGGATGTATGG	353-372	C384c	GCGTCTTCTTCCCGTTCTC	589-608
C309	CCAGAGGATGTCTGGCTGC	357-376	C385c	GCGTCTTCTTCCTGTTCTC	589-608

Probes marked c are complementary to the coding sequence
Probes C302, C305, C307, C308, C321, C322, C325 and C32D correspond to B302, B306, B304, B305, B328, B324, B329 and B322 respectively

The hybridised filter was removed from the tube and rinsed twice in 3x SSC for 10 minutes at room temperature and washed in a solution containing 3M TMAC (tetramethylammonium chloride, 50mM tris-HCl (pH 8.0), 2mM Na_2EDTA, and 0.1% SDS) for 25 minutes in a 59^{o}C shaking water bath.

The air-dried filter was mounted on blotting paper, covered with a piece of plastic wrap and exposed to a Fuji RX x-ray film in a cassette at -70^{o}C. The film was developed after 12-14 hours exposure. If the signal on the film was too weak or strong, it was exposed for a second time (Photograph 4.1).



Photograph 4.1 Autoradiograph - hybridised oligonucleotides appear as dark blots

4.3.7 Assignment of HLA alleles and genotypes

Alleles and genotypes were assigned from the SSO hybridisation patterns using a software package developed in the Human Genetics Group, JCSMR. The package compares the SSO hybridisation patterns to those of all possible genotypes generated by known alleles pre-installed on the HLA typing program. At the current level of polymorphism 99% of all theoretical genotypes are distinguishable by their unique SSO hybridisation patterns. In the rare instance where the results are ambiguous, the program suggests more than one genotype to the sample in question. The program also recognises any unusual SSO patterns generated by previously unknown alleles.

4.4 Statistical Analysis

Gene frequencies and haplotype frequencies were calculated by direct counting using a d-BASE program. Significant linkage disequilibrium was assessed using three criteria: Delta greater than 20 percent, relative Delta greater than 0.7 and chi-squared (χ^2) greater than 3.84.

The Fisher exact test for Hardy-Weinberg equilibrium and linkage disequilibrium in study populations was estimated using GDA32X (Lewis and Zankin 1997) and Arlequin (Excoffier 1996-98) respectively over 17,000 runs. A Bonferroni correction was applied to the resulting probabilities such that for eight populations and three loci (24 tests) the adjusted five percent significance level is p= 0.002. Genetic distances were estimated using Nei's standard genetic distance (D_S) between all pairs of populations (Nei 1972) based on HLA-A gene frequencies and HLA-B-C haplotypes assigned from the linkage disequilibrium data. The resulting distance matrix was converted into a dendrogram using group averaging clustering techniques.

Heterozygosity was estimated from the gene frequency data using the methods outlined by Hartl and Clark (1997). Wright's F statistics were estimated to assess the existence and nature of evolutionary processes causing differentiation between the various study populations (Wright 1921). Specifically we estimated F_{is}, F_{it} and F_{st} using GDA32X. F_{it} measures the reduction in heterozygosity of an individual relative to the overall population. Departure from F_{it} can result from non-random mating (inbreeding) or random genetic drift in subpopulations. Estimates of the magnitude of these influences are measured by the inbreeding coefficient (F_{is}) and fixation index (F_{st}) respectively. Bootstrapping was carried out across all loci to determine the 95 percent confidence interval for assessing the significance of the F statistics.

4.4.1 Statistical Considerations

Ascertainment bias is likely to have occurred due to differential contact of individuals, non-random selection of potential study populations or refusal to participate. For example, in small villages older, sick or pregnant individuals will be less likely to volunteer for bleeding. Also the sample is likely to be biased towards more healthy individuals in society making the discrimination of potential confounders such as malarial pathology complex although inferences based on published malarial surveys for the study populations can be used.

Observation bias may result from systematic differences in the way data was obtained. Examples include inconsistencies in recruitment methods between localities and varying technical competence in venipuncture between personnel and misclassification. Misclassification is particularly difficult to avoid in third world countries such as New Guinea where family relationships are defined differently to Western countries. Parenthood is often unknown either because of widespread adoption practices in which the child loses contact with the biological parents (Morris et al. 1972), polygamy (Shields et al. 1987) or differently defined terms for family relationships. For instance, the term 'uncle' could equally be used to describe your father's brother or a close family friend. Further misclassification may occur because of inaccurate information about the place of birth, particularly among semi-nomadic populations, or inaccuracies in recording statistics such as age because of a statistical digital preference for numbers ending in zero or five in third world countries.

In this study, potential confounders included non-random mating patterns, disease and linkage with other genes. Melanesian populations are often highly inbred due to both consanguinity and small population size. Assortative mating is practiced in many parts of mainland New Guinea and is enhanced by linguistic barriers, malarial distribution and cultural practices. Founder effects are more easily detected in the more recently inhabited parts of Island Melanesia such as New Caledonia. As previously discussed few disease associations have been established involving class I genes although it is likely that pathogens act selectively creating the observed allelic diversity. However, the role of natural selection on HLA loci is not well understood at present. Linkage disequilibrium relationships between HLA class I and other genes may distort the observed relationships between the classical class I genes. Examples include three locus associations and linkage with class II genes, complement or TNF.

4.5 Comparison of B allele binding preferences with those of B*5301

The amino acid binding preferences for the second and ninth peptide binding environments (pockets) for each of the HLA-B alleles detected in the study populations were ascertained using a roadmap for HLA-A, B and C peptide binding specificities developed by Chelvanayagam (1996) and compared to those determined for B*5301 (Hill et al. 1992). The results are presented in the next chapter.

Chapter 5 Results

5.1 Introduction

This chapter presents the results of HLA class I typing in selected New Guinea populations and New Caledonia using PCR-SSO typing techniques. Both allelic and inter-locus haplotypic variations are described. HLA profiles of the Melanesian study populations are compared with other regional populations including Australian Aborigines, Polynesians, Micronesians, Javanese and Chinese. Unless indicated otherwise, all the data for the latter populations mentioned in this and the next chapter are Gao's unpublished results.

5.2 Allelic variation

A total of 8 A alleles, 18 B alleles and 10 C alleles were detected. This contrasts with 6 A alleles, 10 B alleles and 5 C locus specificities previously reported from New Guinea and island Melanesia using serological methods. DNA typing split most of the already known serological specificities from New Guinea and was able to resolve a number of serological 'blanks' particularly at the C locus.

5.2.1 HLA-A locus

DNA typing confirmed the presence of the six HLA-A locus specificities previously detected in these populations using serology: A2, A11, A24, A26, A31 and A34. Two of them, A2 and A24 could be further split into two subtypes each. The others were each represented by a single subtype. This contrasts with multiple subtypes reported from other parts of the world. Eight HLA-A alleles were detected in the study populations: A*0201, A*0206, A*1101, A*2402, A*2407, A*2601, A*3101 and A*3401 using PCR-SSO typing. Three of these alleles: A*1101, A*2402 and A*3401, were predominant with a combined gene frequency ranging from 89% to 99% in the study populations. The gene frequencies for each population are presented in Table 5.1.

A*1101 was detected in all Melanesian study populations at widely varying frequencies. It was predominant at Madang and in the Haruai (50-65%), less prevalent in Wosera and Rabaul (25-40%) and was detected at lower frequencies in samples from the Karimui Plateau, Wanigela and New Caledonia (13-18%). In contrast, Goroka had a very low frequency of A*1101 (~ 2.5%).

A24 could also be split into two subtypes: A*2402 and A*2407. Although A*2402 was detected in all study populations, its frequency varied widely. It was detected at the highest frequency in Goroka and the Karimui Plateau (> 70%), at moderate frequencies at Wosera, Wanigela and New Caledonia (50%-61%) and at lower frequencies in the Haruai and Rabaul (10-30%). In contrast, A*2407 was only detected at Wanigela and Rabaul (3.5-5.7%) and was absent in the other study populations. A*2402 is one of the most common HLA alleles throughout the world, whereas A*2407 is commonly seen in Javanese and Micronesians.

A*3401 also had a wide variation in frequency. It was detected at the highest frequency in Goroka, the Haruai and Rabaul (20-31%), at moderate frequencies in Wosera, Wanigela, Madang and Rabaul (9.7-15.0%) and at low frequency on the Karimui Plateau (4.8%). A*3401 is a common allele in Australian Aborigines (Leinert et al. 1995) and other Oceanic populations.

The study confirmed the proposition that A26 is restricted to Austronesian-speaking populations (Serjeantson 1989). This allele was only found in Rabaul (8.3%), Madang and New Caledonia (<5%). In contrast, A*3101 was detected at low frequencies in all study populations (1-3%) except Goroka where it was absent. Finally, although A2 was virtually absent in the study populations, low frequencies were detected in individuals from New Caledonia, Wanigela and Goroka. A2 split into two subtypes: A*0201, commonly detected throughout the world, and A*0206, a Polynesian marker also commonly detected in Asians (Gao et al. 1997).

5.2.2 HLA-B locus

DNA typing confirmed the presence of the ten alleles previously detected by serology: B13, B18, B27, B35, B39, B48, B56, B60, B61 and B62 (B15), but further split them into subtypes. A total of eighteen B alleles were detected in this study as B15 split into four subtypes; B39 and B40 split into three subtypes; and B27 and B56 each could be split into two subtypes. A previously unidentified allele, B*4010, was also detected.

The gene frequencies for B locus alleles subtyped using PCR-SSO are given in Table 5.2. Of these, B*1301 was distributed throughout all study populations. It was a common allele at Wosera, Madang, Wanigela and Rabaul (13.1-29.3%) but was detected at lower frequencies in other study populations (3.6-7.7%). B*1301 is a common allele in Australian Aborigines and Asian populations as well (Lin et al. 1995).

B15 was commonly detected in all Melanesian study populations except Rabaul. DNA typing split it into four subtypes providing a unique profile for each population. B*1506 was a common allele throughout mainland New Guinea and was detected with greatest frequency in Goroka, the Haruai and Madang (19.1-27.3%), but with much lower frequency in New Caledonia (3.9%). This allele has also been detected in Oriental populations (Lin et al. 1996) and Micronesians but not in Australian Aborigines or Polynesians (Gao et al. 1997). In contrast, the two major B15 alleles in Australian Aborigines and Javanese, B*1521 and B*1525, were detected at much lower frequencies in Melanesians. In particular, B*1521 was detected in Goroka and New Caledonia (5-7%) and at lower frequencies on the Karimui Plateau and Wanigela (1.5-2.5%). B*1525 was confined to Goroka (1.5%), the Haruai (8%) and Wosera (1.5%). Finally, B*1536 was only detected in the Haruai and at Madang (4.5-7.5%). This allele was not been detected in any other population.

In this study, most of the B27 alleles were the Asian subtype B*2704. This allele had the highest frequency in the Karimui (20.6%), but was also commonly detected in Goroka, the Haruai and Wosera (8.8-10%). Lower frequencies were detected at Madang, Wanigela and New Caledonia (3-5%) and it was not detected at Rabaul. Another B27 type, B*2706, commonly seen in Javanese, Southeast Asians and Asian Indians (Gao et al. 1997, Gonzalez-Roces et al. 1997) was detected only at Wanigela (0.8%). B27 is not found in Australian Aborigines and is only rarely detected in other Pacific populations (Gao et al. 1997). B27, however, has been shown in previous serological studies to have the highest gene frequencies in New Guinea populations (Bhatia et al. 1988a).

DNA typing split HLA-B39 into three subtypes: B*3901, B*3903 and B*3906. Of these B*3901 was detected in all populations except Goroka and the Haruai. Gene frequencies ranging from 0.7-2.6% were found in samples from the Karimui Plateau, Wosera and Rabaul, 4.8-6.4% in Madang and New Caledonia and 16.7% in Wanigela. B*3901 is also detected at low frequencies in Australian Aborigines, Polynesians and Micronesians (Gao et al. 1997). B*3903 was only detected at Rabaul (13%). This variant has previously only been reported in South Amerindians (Watkins et al. 1992). Finally, B*3906, a Caucasian allele (Gao et al. 1997), was detected at Wanigela (2.3%) but not in any other Melanesian population. B39 has been reported in coastal Melanesians (Crane et al. 1985) and New Caledonians (Serjeantson 1982, 1983).

High resolution typing also split B40 into three subtypes: B*4001, B*4002 and B*4010. Most populations had both B*4001 and B*4002, but the proportion differed markedly in each population. Of these, B*4001 was detected in all populations except Madang. It was a common allele in Goroka (18.6%) and was detected with moderate gene frequencies in the Karimui, at Rabaul and New Caledonia (8.8-12.5%) and at lower frequencies in the Haruai, Wosera and Wanigela (1.4-5.3%). In contrast, B*4002 was detected in all Melanesian populations and was a major allele in the Karimui, the Haruai and at Wosera (12.7-36.4%) and was detected at lower frequencies (1.8-5.7%) in the remaining

populations. Although both alleles are commonly found throughout the world, B*4002 is common in Australian Aborigines and Micronesians and B*4001 is common in Polynesians (Gao et al. 1997). Finally, B*4010 was detected at low frequencies in Wosera, Wanigela, Madang and New Caledonia (0.7-2.9%) and was a major allele in Rabaul (15.8%). It is also a found in Polynesians and Micronesians but not in Australian Aborigines or Javanese (Gao et al. 1997).

DNA typing confirmed the presence of B56 as a major antigen in Melanesian populations but split it into two subtypes: B*5601 and B*5602. Both alleles were detected in all the study populations and B*5601 was a major allele in all populations (11-35%) except New Caledonia where B*5602 was detected at a higher frequency (24%). B*5602 was detected at much lower frequencies (<10%) in all other populations. The relative proportions of these alleles differentiated Melanesians from other regional populations. B*5602 is a common allele in Australian Aborigines and Polynesians and the only subtype detected in Javanese (Gao et al. 1997).

Only one subtype was detected for serological specificities B18, B35 and B48. These were: B*1801, B*3505 and B*4801 respectively. Low frequencies of B*1801 were detected at Wanigela, Madang and New Caledonia (0.9-1.9%) and it was absent in other study populations. B*1801 is commonly detected in Southeast Asian populations, including Javanese (Gao et al. 1997), but is absent in Australian Aborigines and other Oceanic populations.

B*3505, a common allele in Javanese and Micronesians, was detected at low frequency in Wanigela (2.3%) only. B*3505 is also detected in South Amerindians (Belich et al. 1992, Watkins et al. 1992). Finally B*4801 was detected at low frequencies in all coastal Melanesian populations (1.3-2.3%) and has also been detected with a relatively high frequency in Polynesians (Gao et al. 1997). B48 is a common allele in Eskimos and Amerindians (Watkins et al. 1992) but is rarely detected in other populations.

5.2.3 HLA-C locus

DNA typing confirmed the presence of specificities previously reported by serology: Cw1, Cw3, Cw4 and Cw7, split them further into subtypes, and resolved the identity of the large proportion of serological 'blanks' reported at the C locus. Altogether 10 officially designated C alleles were detected in the study populations: Cw*0102, Cw*0303, Cw*0304, Cw*0401, Cw*0403, Cw*0702, Cw*0801, Cw*1202, Cw*1203 and Cw*1502. Their gene frequencies are presented in Table 5.3. In addition, a novel Cw12 allele was identified in three individuals from Goroka. The study did not confirm the presence of Cw6 in Melanesia.

Cw3 was present in all study populations and comprised two subtypes: Cw*0303 and Cw*0304. Cw*0303 was detected in all populations except at Madang. Although it was a common allele at Goroka, the Karimui and Rabaul (16.1-28.1%) it was only detected at low frequencies in the rest of the study populations (<5%). Cw*0303 has also been recorded throughout Southeast Asia and in Australian Aborigines and Polynesians (Bodmer et al. 1997b, Gao et al. 1997). In contrast, Cw*0304 was detected in all populations except Goroka. It was a common allele in the Karimui (18.1%) and Wosera (22.3%) and detected with moderate frequencies at Wanigela, Madang and New Caledonia (5-10%) and at lower frequencies in the Haruai and at Rabaul (1.8-3.3%). Although Cw*0304 is virtually absent in Australian Aborigines, it is a common allele in mainland Asia (Gao and Matheson 1996), Southeast Asia (Bodmer et al. 1997b) and Polynesia (Gao et al. 1997).

Cw4 was also present in all populations and split into two subtypes: Cw*0401 and Cw*0403. Of these Cw*0401 was commonly detected in the Haruai, at Wosera, Wanigela and New Caledonia (14.8-20.8%), at moderate frequencies in the Karimui and Madang (5-10%) and at low frequencies at Goroka and Rabaul (<5%). In contrast, Cw*0403 was predominant at Goroka (33.7%), the Haruai (45.9%) and Madang (25.0%) and was detected at moderate frequency at Wanigela (16.0%), the Karimui (11.9%), Rabaul (8.9%) and Wosera (8.0%). Both subtypes are common in Australian Aborigines and other Oceanic populations (Gao et al. 1997).

DNA typing showed that Cw1 and Cw7 were each represented by a single DNA subtype: Cw*0102 and Cw*0702 respectively. Cw1 was a common allele at Goroka (30.2%), among the Haruai (18.9%) and the Karimui (20.0%) and in New Caledonia (37.8%) and was found at lower frequencies in lowland and other coastal populations (7.2-14.7%). Cw*0102 is also commonly found in Australian Aborigines and other populations of the Pacific (Gao et al. 1997).

Cw*0702 was present in all populations except the Haruai. It was detected with highest frequencies at Madang and Rabaul (29.3% and 57.1%), moderate frequencies in the Karimui, Wanigela and New Caledonia (11.1%-12.5%) and at low frequency at Goroka (3.5%). Cw7 has previously been described as a coastal antigen (3.6%) (Crane et al. 1985) although it was later reported from the Karimui Plateau (Bhatia et al. 1988).

In serological studies 'blanks' account for more than 50 percent of the HLA-C gene frequencies in coastal Melanesian populations (Crane et al. 1985) and 14 percent in highlanders (Bhatia et al. 1986). The high resolution DNA typing performed in this study was able to resolve completely the HLA-C polymorphism in the study populations and found these serological 'blanks' mainly comprised subtypes of Cw*08, Cw*12 and Cw*15. Of these, Cw*0801 was detected at low frequencies only at Wanigela, Madang and New Caledonia (1.8-2.6%). Cw*12 split into two subtypes, Cw*1202 and Cw*1203. Of these, Cw*1202 was detected in all populations except Goroka and Rabaul. Wosera had the highest frequency (11.6%) and it was detected at slightly lower frequencies in the Haruai and Madang (6.0-8.2%) and at low frequencies in the Karimui, Wanigela and New Caledonia (3.1-4.9%). Cw*1203 is found in Japanese, northern Thais (Bodmer et al. 1997b) and Polynesians but has not been detected in Australian Aborigines (Gao et al. 1997). In this study, low frequencies of Cw*1203 (1.2-3.8%) were detected in all study populations except the highland fringe populations, which lacked this antigen, and Wosera, where it was detected with a much higher frequency (11.6%). Finally, Cw*1502 was detected with a wide variation in all study populations. Cw*1502

was a common allele at Wosera (23.2%) and found at moderate frequencies in the Karimui, the Haruai and New Caledonia (5.6-10.0%) and low frequencies in the remaining study populations (1.4-3.5%). Cw*1502 is a common antigen in other Pacific populations (Gao et al. 1997).

5.3 Alleles Associated with Austronesian-speaking Populations

Nine alleles that are commonly detected in Southeast Asians and/or other Pacific populations were detected at low frequencies exclusively in Austronesian-speaking coastal New Guinea and island Melanesian study populations. These were A*2407 and 2601; B*1801, B*2706, B*3505, B*3903, B*4010, B*4801; and Cw*0801. None of these alleles have been detected in Wosera, a lowland non-Austronesian-speaking population or in Australian Aborigines (Gao et al. 1997). In addition a further six alleles: A*0201, A*0206, B*1536, B*3901, B*3906 and Cw*1203 were sporadically detected in various populations. All of these alleles are commonly found in other neighbouring Austronesian-speaking populations, including Polynesians, Micronesians and Javanese.

In the four Austronesian-speaking Melanesian populations (Madang, Wanigela, Rabaul and New Caledonia) these alleles accounted for 2.6-11.8 % of the gene frequency in the A locus, 11.5-31.9 % in the B locus and 1.7-13.2 % in the C locus. In contrast, in the non-Austronesian-speaking populations (Goroka, Karimui, Haruai and Wosera) they accounted for 0-2.6 % in the A locus, 0-4.6% in the B locus and 0-1.2 % in the C locus.

5.4 Linkage Disequilibrium Between class I Loci

DNA typing was able to detect many two-locus haplotypes previously unrecognisable by serology, providing a more accurate estimate of linkage disequilibria between the three class I loci. Tables 5.4, 5.5 and 5.6 show estimates of haplotype frequencies and coefficients of linkage disequilibrium for the statistically significant two-locus associations found in the study populations. The

relative delta values are presented in Appendix 1. Twenty two B-C haplotypes, eight A-B haplotypes and eight A-C haplotypes were detected that revealed statistically significant associations.

5.4.1 HLA B-C

As expected, stronger linkage disequilibrium was observed between the B and C alleles than between A and B or A and C alleles since these loci are more closely linked (Bodmer and Bodmer 1978). For example, B*1301 was found in association with three Cw alleles: Cw*0304, Cw*0401 and Cw*1203. Serology had detected B13-Cw4 in Australian Aborigines (Hay 1986) and throughout Melanesia (Serjeantson 1982, Bhatia et al. 1984, 1986, 1988, 1989, Crane et al. 1985). The other B13 haplotypes had a more restricted distribution. B*1301-Cw*0304 was only detected at Wosera and Madang and B*1301-Cw*1203 was only found at Rabaul.

Similarly, B62-Cw4 comprised three haplotypes with significant associations: B*1506-Cw*0401, B*1521-Cw*0403 and B*1525-Cw*0403. Of these, B*1506-Cw*0401 was detected in all the study populations except Rabaul; and is not detected in other regional populations (Gao et al. 1997). B*1521-Cw*0403 was found at Goroka, the Karimui Plateau, Wosera and New Caledonia. It is also found in Australian Aborigines, but not in other regional populations (Gao et al. 1997). The distribution of B*1525-Cw*0403 associations was restricted to the Haruai and Wosera. It is not detected in Australian Aborigines or other Oceanic populations (Gao et al. 1997). A fourth B62 haplotype, B*1536-Cw*0102, was restricted to the Haruai and Madang.

B*2704 associates significantly with three different Cw alleles; Cw*0102, Cw*0304 and Cw*1202. Of these, B*2704-Cw*12022, a common Asian haplotype (Gao and Matheson 1996), was detected in all study populations, except Goroka and Rabaul, B*2704-Cw*0304 was restricted to the Karimui and B*2704-Cw*0102 to Goroka.

DNA typing detected two strong Cw significant associations with B*3901. These were B*3901-Cw*0702 found in the Karimui, at Wanigela and in New Caledonia, and B*3901-Cw*1203 found in Wanigela and Madang. B*3901-Cw*0702 is found in Australian Aborigines, Polynesians and Micronesians whereas B*3901-Cw*1203 is detected in Australian Aborigines and Micronesians.

B*4001 associated with three Cw alleles in the study populations: Cw*0303 found in Goroka, the Karimui and the Haruai; and Cw*0304 and Cw*0401 in the Haruai and New Caledonians. B*4001-Cw*0303 is found in Australian Aborigines but not other Oceanic populations (Gao et al. 1997). B*4001-Cw*0304 is commonly found in Caucasians, mainland Asians and West Samoans and B*4001-Cw*0401 is commonly detected in Micronesians and Polynesians. B*4002 was found in three different significant associations in the study populations: with Cw*0102 in the Haruai and Australian Aborigines, with Cw*0303 in the Karimui and Wanigela and with Cw*1502 in all study populations, except Madang. It is also found in Australian Aborigines, Micronesians and Polynesians. B*4010 was found with significant linkage disequilbrium in association with Cw*0403. It was detected in all coastal/island study populations and Wosera and is also found in Polynesians and Micronesians. B*4010-Cw*0702 was detected at Rabaul, but not in significant linkage disequilbrium, suggesting that it may have a local origin.

DNA typing showed that as well as being comprised of two subtypes, B56 associated with three Cw alleles to form four haplotypes in significant linkage disequilibrium. These were: B*5601-Cw*0102, B*5601-Cw*0401, B*5601-Cw*0702 and B*5602-Cw*0102. Of these, the two B56-Cw1 haplotypes were distributed most widely. B*5601-Cw*0102 was found in all study populations except Madang and Rabaul. It is also found in Australian Aborigines, Kiribati and Polynesia. B*5602-Cw*0102 was found in all study populations except the Haruai and Madang and is a common haplotype in Australian Aborigines and Micronesians. Of the remaining B56 haplotypes, B*5601-Cw*0401 associations were only found

in linkage disequilibrium in the Haruai, whereas B*5601-Cw*0702 was found at Goroka and Madang. Both haplotypes are also found in Australian Aborigines.

Of the remaining haplotypes B*1801-Cw*0702 and B*3505-Cw*0401 were only found in Wanigela, and B*4010-Cw*0403 at Wosera and Rabaul, whereas B*4801-Cw*0801 was detected at Madang, Rabaul and New Caledonia. B*1801-Cw*0702 has also been detected in Javanese, whereas B*3505-Cw*0401 is found in Javanese and Micronesians. B*4010-Cw*0403 is commonly detected in Micronesians and B*4801-Cw*0801 is found in mainland Asians, Micronesians and Polynesians.

5.4.2 HLA-A-B

A total of eight A-B haplotypes with significant linkage disequilibrium were detected in the study populations. No statistically significant A-B associations were detected at the Karimui or at Wosera. DNA typing split most A alleles into multiple haplotypes, all of which, except one, were restricted to one or two study populations. For example, A*1101 formed an association with three different B alleles, each restricted to a single study population. These were: A*1101-B*1301 only found in Madang, A*1101-B*4001 and A*1101-B*4010 only found at Rabaul.

A*2402 formed associations with two B alleles; B*4001 and B*5601. The former was found in Goroka and New Caledonia, whilst the latter was found in Goroka, Madang and Wanigela. A*2402-B*4001 is also detected in Polynesians and Micronesians, whereas A*2402-B*5601 is found in Micronesians. Serology had detected A24-B60 in the Hagahai (Bhatia et al. 1989) but not in other Melanesian populations.

DNA typing showed that A*3101 was only associated with B*1506 with significant linkage disequilibrium at Madang. This association is not found in other regional populations. Similarly, high resolution typing showed that B*3401 was associated with B*1525, B*4002 and B*4010. The first two were found in the Haruai and Rabaul, whilst the latter was only found at Madang. A34-B56 is detected in north

coastal New Guineans and the Watut but not in the Asaro (Crane et al. 1985) using serology. A*3401-B*4002 is also found in Micronesia and Polynesia but not in Australian Aborigines or Southeast Asians. None of the other haplotypes are detected in other regional populations (Gao et al. 1997).

Finally, A*2407- B*3505 was detected at Wanigela, the only population with these alleles, although the association was not statistically significant. This haplotype is commonly found in Micronesians and sporadically in Javanese.

5.4.3 HLA-A-C

A total of eight statistically significant A-C haplotype associations were detected. No A-C haplotypes were detected at Wosera, Wanigela, Rabaul or New Caledonia and all but one of these were restricted to a single population. In particular, A*1101 was in significant linkage disequilibrium with Cw*0304 at Madang; Cw*0401 in the Haruai and Madang; Cw*1202 in the Karimui and Cw* 1502 in the Haruai. Whereas A*2402 was found in association with Cw*0303 in Goroka; Cw*0304 in the Haruai; and Cw*1502 in the Karimui. A*3101-Cw*0403 was detected in Madang. None of these associations are found in other regional populations.

5.5 Genetic Interrelationships Between New Guinea Populations

5.5.1 Population genetic structure

As expected the B locus was more heterozygous than other class I HLA loci since the B locus has more variation in the peptide binding region than other class I loci, reflected in the larger range of alleles detected (9-13). Observed heterozygosity at B ranged from 0.727 at Madang to 0.939 at Wanigela. The C locus which had a similar number of alleles (8-11) was only slightly less heterozygous with observed heterozygosity ranging from 0.607 at Rabaul to 0.929 at Wosera. In contrast, much lower levels of heterozygosity were detected in the A locus where observed

heterozygosity ranged from 0.410 at Goroka to 0.650 at Wosera. These lower values reflect the smaller number of alleles (4-7) detected in the study populations. The theoretical and observed mean heterozygosity for all populations was almost identical.

Exact tests do not provide evidence of departure from Hardy Weinberg equilibrium at any of the loci. However, exact tests for linkage disequilibrium did support the existence of linkage disequilibrium, after correcting for multiple tests, between the B and C loci for all populations except Rabaul, between A and C loci for Haruai, Wosera and Karimui and between A and B for Haruai, Wosera and Madang.

Wright's F statistics were estimated to assess the existence and nature of evolutionary processes causing differentiation between the various study populations (Wright 1921). The inbreeding coefficient (F_{is}) was not significant showing no deviation from random mating (i.e. inbreeding) within any of the study populations. However, the fixation index (F_{st}) was statistically significant indicating the study populations were well differentiated from each other. This may be due to random genetic drift or natural selection.

5.5.2 Genetic distances

Genetic distances were calculated based on HLA-A gene frequencies and B-C haplotypes to compare the study populations with Asians, Australian Aborigines and other Oceanic populations. A matrix of these values is shown at Table 5.9. This data was converted to a dendogram presented in Figure 5.1. In general all regional populations were well separated. Within Melanesia, mainland New Guinea populations clustered together whilst Rabaul formed a separate group. New Caledonians showed a closer affinity to Polynesians and Micronesians.

Table 5.1: Gene Frequencies of HLA-A alleles in Melanesian populations

	Goroka n=38	**Karimui Plateau** n=84	**Haruai** n=58	**Wosera** n=36	**Wanigela** n=70	**Madang** n=57	**Rabaul** n=42	**New Caledonia** n=41
A*0201	0.0	0.0	0.0	0.0	0.0285	0.0	0.0	0.0244
A*0206	0.0263	0.0	0.0	0.0	0.0214	0.0	0.0	0.0
A*1101	0.0263	0.1786	0.5431	0.3750	0.1786	0.6404	0.2738	0.1341
A*2402	0.7368	0.7441	0.1379	0.5139	0.5786	0.1579	0.2864	0.6098
A*2407	0.0	0.0	0.0	0.0	0.0571	0.0	0.0350	0.0
A*2601	0.0	0.0	0.0	0.0	0.0	0.0263	0.0833	0.0122
A*3101	0.0	0.0298	0.0172	0.0139	0.0143	0.0526	0.0119	0.0244
A*3401	0.2105	0.0476	0.3017	0.0972	0.1214	0.1228	0.3095	0.1463
Others	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0488
Total	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

n = number of individuals examined
Others comprise typical Caucasian alleles such as A*2501

Table 5.2 Gene Frequencies of HLA-B alleles in Melanesian populations

	Goroka n=35	**Karimui Plateau** n=80	**Haruai** n=55	**Wosera** n=70	**Wanigela** n=66	**Madang** n=55	**Rabaul** n=38	**New Caledonia** n=52
B*1301	0.0571	0.0688	0.0364	0.2929	0.1742	0.1909	0.1316	0.0769
B*1506	0.2286	0.1000	0.2727	0.0929	0.1288	0.1909	0.0	0.0385
B*1521	0.0714	0.0250	0.0	0.0	0.0152	0.0	0.0	0.0577
B*1525	0.0143	0.0	0.0818	0.0143	0.0	0.0	0.0	0.0
B*1536	0.0	0.0	0.0455	0.0	0.0	0.0727	0.0	0.0
B*1801	0.0	0.0	0.0	0.0	0.0152	0.0091	0.0	0.0192
B*2704	0.1000	0.2063	0.0909	0.0877	0.0379	0.0545	0.0	0.0385
B*2706	0.0	0.0	0.0	0.0	0.0076	0.0	0.0	0.0
B*3505	0.0	0.0	0.0	0.0	0.0227	0.0	0.0	0.0
B*3901	0.0	0.0187	0.0	0.0071	0.1667	0.0636	0.0263	0.0481
B*3903	0.0	0.0	0.0	0.0	0.0	0.0	0.1316	0.0
B*3906	0.0	0.0	0.0	0.0	0.0227	0.0	0.0	0.0
B*4001	0.1857	0.0875	0.0455	0.0143	0.0530	0.0	0.0921	0.1250
B*4002	0.0571	0.2562	0.1273	0.3643	0.0455	0.0182	0.0395	0.0288
B*4010	0.0	0.0	0.0	0.0071	0.0152	0.0182	0.1579	0.0288
B*4801	0.0	0.0	0.0	0.0	0.0227	0.0182	0.0132	0.0192
B*5601	0.2286	0.1562	0.2091	0.1143	0.1667	0.3455	0.3158	0.1923
B*5602	0.0857	0.0375	0.0909	0.0143	0.0682	0.0091	0.0658	0.2404
Others	0.0	0.0500	0.0	0.0	0.0380	0.0	0.0	0.0864
Total	*1.0000*	*1.0000*	*1.0000*	*1.0000*	*1.0000*	*1.0000*	*1.0000*	*1.0000*

n = number of individuals examined
Others comprise typical Caucasian alleles such as B*0702

Table 5.3: Gene Frequencies of HLA-C alleles in Melanesian populations

	Goroka n=43	**Karimui Plateau** n=80	**Haruai** n=61	**Wosera** n=56	**Wanigela** n=72	**Madang** n=58	**Rabaul** n=28	**New Caledonia** n=45
Cw*0102	0.3023	0.2000	0.1885	0.1071	0.1319	0.1466	0.0714	0.3778
Cw*0303	0.2209	0.2812	0.0164	0.0179	0.0486	0.0	0.1607	0.0333
Cw*0304	0.0	0.1812	0.0328	0.2232	0.0833	0.0948	0.0179	0.0556
Cw*0401	0.0465	0.0688	0.1475	0.1696	0.2083	0.0948	0.0357	0.1556
Cw*0403	0.3372	0.1187	0.4590	0.0804	0.1597	0.2500	0.0893	0.1111
Cw*0702	0.0349	0.1187	0.0	0.0536	0.1250	0.2931	0.5714	0.1111
Cw*0801	0.0	0.0	0.0	0.0	0.0178	0.0259	0.0	0.0222
Cw*1202	0.0	0.0312	0.0820	0.1161	0.0486	0.0603	0.0	0.0444
Cw*1203	0.0116	0.0	0.0	0.0	0.1319	0.0172	0.0357	0.0222
Cw*1502	0.0349	0.1000	0.0738	0.2321	0.0139	0.0086	0.0179	0.0556
Others	0.0	0.0	0.0	0.0	0.0208	0.0086	0.0	0.0111
Total	*1.0000*	*1.0000*	*1.0000*	*1.0000*	*1.0000*	*1.0000*	*1.0000*	*1.0000*

n= number of individuals examined
Others comprise typical Caucasian alleles

Table 5.4 Two-locus B-C haplotypes showing significant linkage disequilibrium in Melanesian populations

	Goroka				Haruai				Wosera				Karimui				Madang				Wanigela				Rabaul				New Caledonia			
	n=35				n=55				n=109				n=74				n=55				n=65				n=18				n=40			
	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2
B*1301-Cw*0304	-	-	-	-	/	/	/	/	**22.7**	**12.7**	**0.8**	**26**	/	/	/	/	**9.5**	**7.7**	**1**	**43.6**	/	/	/	/	2.8	2.6	1	2.3	/	/	/	/
B*1301-Cw*0401	**4.4**	**4.1**	**1**	**35.1**	2.6	2	0.65	5.8	8.3	2.9	0.3	2.3	**5.6**	**5.2**	**0.9**	**99.4**	**9.5**	**7.7**	**1**	**43.6**	**13.9**	**10.8**	**0.7**	**65.7**	/	/	/	/	**6.5**	**5.5**	**1**	**24.9**
B*1301-Cw*1203	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	0.7	0.4	0.2	0	/	/	/	/	**5.7**	**5.2**	**1**	**12.3**	/	/	/	/
B*1506-Cw*0403	**19.9**	**11.5**	**0.7**	**19.4**	28.2	13.9	0.9	37.8	**6.3**	**5.8**	**1**	**53.6**	**9.9**	**8.7**	**0.9**	**107**	**14.7**	**11.6**	**1**	**64.5**	**14.1**	**11.7**	**1**	**98.6**	/	/	/	/	**2.5**	**2.2**	**1**	**6.9**
B*1521-Cw*0403	**7.4**	**4.9**	**1**	**7.7**	-	-	-	-	-	-	-	-	**2.7**	**2.4**	**1**	**23.9**	-	-	-	-	0.6	0.4	0.3	0	-	-	-	-	**7.8**	**6.8**	**1**	**36.1**
B*1525-Cw*0403	1.4	0.9	1	0.1	**8.5**	**4.5**	**1**	**9.1**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*1536-Cw*0102	-	-	-	-	**2.8**	**2.3**	**1**	**9.9**	-	-	-	-	-	-	-	-	**5.4**	**4.4**	**0.7**	**20.7**	-	-	-	-	-	-	-	-	-	-	-	-
B*1801-Cw*0702	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.9	0.7	1	0.3	/	/	/	/	-	-	-	-	/	/	/	/
B*2704-Cw*0102	**7.4**	**5**	**1**	**8.6**	/	/	/	/	/	/	/	/	/	/	/	/	1.3	0.5	0.1	0	2.1	1.5	0.5	3.4	-	-	-	-	1.9	0.7	0.3	0
B*2704-Cw*0304	-	-	-	-	0.7	0.5	0.2	0	/	/	/	/	**16.1**	**13.3**	**0.9**	**105.6**	1.5	1	0.2	0.6	1.4	1.1	0.3	2.2	-	-	-	-	/	/	/	/
B*2704-Cw*1202	-	-	-	-	**8.5**	**7.7**	**1**	**86.3**	**8.9**	**7.8**	**1**	**45.5**	**3.4**	**2.7**	**1**	**15.9**	**3.7**	**3.4**	**0.8**	**41.3**	2.3	2.2	0.6	28.7	-	-	-	-	**3.8**	**3.7**	**1**	**55.1**
B*3505-Cw*0401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.3	1.9	1	9.2	-	-	-	-	-	-	-	-
B*3901-Cw*0702	-	-	-	-	-	-	-	-	/	/	/	/	**2**	**2**	**1**	**102.3**	4	2.2	0.5	2.8	8.6	6.6	0.6	34.9	5.7	2.7	1	0.5	2.4	2	0.6	4.4
B*3901-Cw*1203 #	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.8	1.7	1	15.8	6.5	4.4	0.4	16.6	/	/	/	/	/	/	/	/
B*4001-Cw*0303	**18.8**	**13.7**	**0.9**	**39.2**	1.8	1.7	1	23.1	/	/	/	/	**8.5**	**5.9**	**1**	**28.1**	-	-	-	-	0.6	0.3	0.1	0	2.8	2.6	1	2.3	2.4	1.9	0.6	3.5
B*4001-Cw*0304 #	-	-	-	-	1.8	1.7	0.6	13.8	1.2	0.9	1	0.3	/	/	/	/	-	-	-	-	2.1	1.7	0.3	5.7	/	/	/	/	3.6	2.8	0.5	5.9
B*4001-Cw*0401 #	/	/	/	/	/	/	/	/	/	/	/	/	1	0.4	0.1	0	-	-	-	-	2.7	1.7	0.4	3	/	/	/	/	5.9	4.1	0.4	7.2
B*4002-Cw*0702	/	/	/	/	-	-	-	-	**7.6**	**4.9**	**1**	**9.4**	/	/	/	/	0.6	0.1	0.1	0.5	/	/	/	/	2.8	1.3	1	0	/	/	/	/
B*4002-Cw*1502	**2.9**	**2.7**	**1**	**18.2**	**7.5**	**6.4**	**0.9**	**45.5**	**21.2**	**12.3**	**0.8**	**25.8**	**8.9**	**6.3**	**0.8**	**29.1**	0.9	0.9	1	13.2	1.6	1.5	1	22.6	**2.8**	**2.7**	**1**	**8.7**	**2.5**	**2.4**	**1**	**21**
B*4010-Cw*0403	-	-	-	-	-	-	-	-	1.2	1.1	1	2.6	-	-	-	-	1.8	1.4	1	3.5	0.6	0.4	0.3	0	**8.7**	**6.8**	**1**	**7.8**	**2.5**	**2.2**	**1**	**6.9**
B*4801-Cw*0801	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.8	1.8	1	40.2	**2.3**	**2.3**	**1**	**66.3**	-	-	-	-	**2.5**	**2.5**	**1**	**44.6**
B*5601-Cw*0102	13..5	6.2	0.4	7	7	3.4	0.2	5.4	**8.7**	**6.9**	**0.7**	**23.2**	**13.7**	**10.7**	**0.9**	**77.7**	6.4	1.7	0.2	1.3	9.2	6.5	0.5	29.6	/	/	/	/	13.5	7.2	0.6	9.9
B*5601-Cw*0401	/	/	/	/	**12.2**	**8.9**	**0.7**	**34.8**	5.3	3	0.2	2.7	1.3	0.4	0.1	0	/	/	/	/	/	/	/	/	-	-	-	-	/	/	/	/
B*5601-Cw*0702	**4.4**	**3.4**	**1**	**6.9**	-	-	-	-	1.6	0.4	0.1	0	/	/	/	/	**21.6**	**12.3**	**0.7**	**37.5**	/	/	/	/	28.5	6.1	0.3	1.3	2.4	0.3	0	0
B*5602-Cw*0102	**7.4**	**5**	**1**	**8.6**	/	/	/	/	1.2	1.1	1	1.5	**3.3**	**2.5**	**0.7**	**11**	0.9	0.8	1	1.1	3.6	2.6	0.5	8.8	**8.7**	**7.7**	**1**	**17.2**	17.5	1.2	0.9	19.8

hf%: haplotype frequency D%: Delta value rD: relative delta value
Dash indicates at least one of these alleles does not occur in this population
Slash indicates this haplotype was not detected in this population
Bold indicates HLA haplotypes with significant linkage disequilibrium (where $\chi^2 > 3.84$, D% > 2.0, relative D > 0.70)
Hash indicates HLA haplotypes where significant linkage disequilibrium is found in other regional populations

Table 5.5 Two-locus A-B haplotypes showing significant linkage disequilibrium in Melanesian populations

	Goroka				Haruai				Wosera				Karimui				Madang				Wanigela				Rabaul				New Caledonia			
	n=32				n=55				n=40				n=78				n=54				n=67				n=37				n=40			
	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2
A*1101-B*1301	/	/		/	3.8	1.8	1	2.1	/	/	/	/	/	/	/	/	**18.4**	**6.1**	**1**	**10.4**	4.7	1.6	0.1	1.6	/	/	/	/	3.7	3.1	0.68	9.4
A*1101-B*4001	/	/		/	/	/	0.7	/	/	/	/	/	/	/	/	/	-	-	-	-	/	/	/	/	6.2	4	0.6	4.3	/	/	/	/
A*1101-B*4010	-	-		-	-	-	-	-	1.6	0.9	1	0	-	-	-	-	1.9	0.6	1	0.1	0.6	0.3	0.3	0	**15.2**	**10.1**	**0.7**	**17.6**	/	/	/	/
A*2402-B*3505 #	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.4	0.9	1	0.7	-	-	-	-	-	-	-	-
A*2402-B*4001	**20.9**	**5.2**	**1**	**4.1**	**3.7**	**3.1**	**0.7**	**14.4**	1.6	0.8	1	0	/	/	/	/	-	-	-	-	3.6	0.2	0.1	0	/	/	/	/	**12**	**5.4**	**1**	**6.9**
A*2402-B*5601	**20.9**	**5.2**	**1**	**4.1**	/	/	/	/	/	/	/	/	12.2	0.4	0.1	0	**12.1**	**6.9**	**0.7**	**5.5**	**15.1**	**4.7**	**0.7**	**7.1**	/	/	/	/	/	/	/	/
A*3101-B*1506	-	-		-	/	/	/	/	/	/	/	/	/	/	/	/	**5.7**	**4.9**	**1**	**30.4**	/	/	/	/	-	-	-	-	/	/	/	/
A*3401-B*1525	/	/		/	**8.7**	**6**	**1**	**19.9**	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*3401-B*4002	/	/		/	**10.1**	**6.2**	**0.7**	**15**	4.8	0.9	0.1	0	/	/	/	/	/	/	/	/	/	/	/	/	3.3	2.4	1	2.3	/	/	/	/

hf%: haplotype frequency D%: delta value rD: relative delta value
Dash indicates that at least one of these alleles does not occur in this population
Slash indicates this haplotype was not detected in this population
Bold indicates HLA haplotypes with significant linkage disequilibrium (where $\chi^2 > 3.84$, $D\% > 2.0$, relative $D > 0.70$)
Hash indicates HLA haplotypes where significant linkage disequilibrium is found in other regional populations

Table 5.6 Two-locus A-C haplotypes showing significant linkage disequilibrium in Melanesian populations

	Goroka				Haruai				Wosera				Karimui				Madang				Wanigela				Rabaul				New Caledonia			
	n=32				n=54				n=40				n=78				n=54				n=67				n=37				n=40			
	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2	hf%	D%	rD	χ2
A*1101-Cw*0304	-	-	-	-	/	/	/	/	/	/	/	/	/	/	/	/	**8.2**	**3.1**	**1**	**4.5**	2.1	0.8	0.1	0.2	/	/	/	/	/	/	/	/
A*1101-Cw*0401	/	/	/	/	**17**	**8**	**1**	**19.1**	8.5	0	0	0	/	/	/	/	**9.2**	**3.4**	**1**	**5.3**	3.7	0.4	0	0.1	3.6	2.7	1	3	6.3	4.1	0.3	5.7
A*1101-Cw*1202	-	-	-	-	5.2	0.5	0.1	0	5.2	1.9	0.4	0.5	**3.3**	**2.6**	**1**	**15.4**	/	/	/	/	0.9	0.1	0	0.2	-	-	-	-	/	/	/	/
A*1101-Cw*1502	1.3	1.2	0.5	2.1	**8.1**	**3.8**	**1**	**7.2**	10.5	1.1	0.1	0.2	/	/	/	/	0.9	0.3	1	0.1	0.6	0.3	0.3	0	/	/	/	/	2.5	1.9	0.5	2.4
A*2402-Cw*0303	**22.2**	**6.2**	**1**	**6.8**	0.7	0.5	0.3	0	/	/	/	/	25.4	0.6	0.1	0.2	/	/	/	/	3.3	0	0	0.1	/	/	/	/	4.1	1.9	1	1.2
A*2402-Cw*0304	-	-	-	-	**3.5**	**3**	**1**	**19.2**	26.4	10.8	0.6	12.3	13.8	0.8	0.2	0.1	/	/	/	/	/	/	/	/	1.8	1.1	1	0.1	4	0.3	0.1	0.1
A*2402-Cw*1502	/	/	/	/	/	/	/	/	/	/	/	/	**10.8**	**2.5**	**1**	**4.2**	0.9	0.7	1	1	1.5	0.6	1	0.2	1.8	1.1	1	0.1	/	/	/	/
A*3101-Cw*0403	-	-	-	-	/	/	/	/	/	/	/	/	/	/	/	/	**5.4**	**4.2**	**1**	**19**	/	/	/	/	/	/	/	/	/	/	/	/

hf%: haplotype frequency D%: delta value rD: relative delta value
Dash indicates at least one of these alleles does not occur in this population
Slash indicates these haplotypes were not detected in this population
Bold indicates HLA haplotypes with significant linkage disequilibrium (where $\chi^2 > 3.84$, D% > 2.0, relative D > 0.70)

	A locus			B locus			C locus		
	k	Hexp	Hobs	k	Hexp	Hobs	k	Hexp	Hobs
Goroka	4	0.411	0.41	9	0.837	0.886	8	0.741	0.791
Haruai	4	0.586	0.583	10	0.753	0.873	8	0.833	0.77
Wosera	4	0.595	0.65	9	0.837	0.871	7	0.719	0.929
Karimui	4	0.411	0.452	10	0.843	0.85	8	0.818	0.85
Madang	5	0.546	0.492	11	0.794	0.727	10	0.807	0.69
Wanigela	7	0.614	0.577	10	0.883	0.939	11	0.868	0.819
Rabaul	6	0.739	0.771	9	0.825	0.844	8	0.631	0.607
New Caledonia	7	0.585	0.548	13	0.865	0.844	11	0.798	0.778
Mean	5	0.561	0.56	10	0.83	0.854	9	0.777	0.779

Table 5.7: Heterozygosity Data

	Fis	Fit*	Fst*	Boundary	Fis	Fit	Fst
A locus	0.0056	0.1624	0.1577	Upper	0.0056	0.1624	0.1577
B locus	-0.0164	0.0563	0.0716	Lower	-0.0164	0.0563	0.0716
C locus	0.0011	0.1012	0.1002	No Reps	999	999	999
Overall	**-0.0044**	**0.1016**	**0.1055**	**CI (realised)**	**95.1951**	**95.1951**	**95.1951**

Asterisk indicates statistically significant variance ie where upper and lower boundaries cross zero

Table 5.8: Analysis of variance and bootstrapping over loci to determine 95% confidence interval

Locus	Goroka	Haruai	Wosera	Karimui	Madang	Wanigela	Rabaul	New Caledonia
HLA-A	0.1805	0.8609	0.0061	0.1989	0.2661	0.6738	0.4838	0.7667
HLA-B	0.5369	0.1743	0.5167	0.5456	0.5025	0.4116	0.0883	0.285
HLA-C	0.4186	0.9789	0.3818	0.3233	0.1245	0.6811	0.1192	0.6746

Table 5.9 Exact test for Hardy Weinberg equilibrium (Bonferroni correction applied)

Locus	Goroka	Haruai	Wosera	Karimui	Madang	Wanigela	Rabaul	New Caledonia
HLA A-B	0.4144	**0**	**0**	0.426	**0**	0.7771	0.0894	0.0062
HLA A-C	0.3256	**0**	**0**	**0**	0.0023	0.9047	0.1684	0.2905
HLA B-C	**0**	**0**	**0**	**0**	**0**	**0**	0.0045	0

Table 5.10 Exact test for linkage disequilibrium (Bonferroni correction applied)

Bold indicates significant linkage disequilibrium

	Wosera	Karimui	Harual	Wanigela	Goroka	New Caledonia	Madang	New Britain	Kiribati	Nauru	Niue	Raratonga	West Samoa	North China	South China	Java	Kimberley	Groote Eylandt	Yuendumu	Cape York
Wosera																				
Karimui	0.27238																			
Harual	0.46402	0.67962																		
Wanigela	0.46586	0.564	0.67405																	
Goroka	0.46619	0.22967	0.77797	0.4283																
New Caledonia	0.48245	0.31957	0.6707	0.5565	0.59558															
Madang	0.61946	0.6604	0.21608	0.66973	0.86151	0.91477														
New Britain	1.12046	0.85574	0.92769	1.02586	1.47121	0.77245	0.49338													
Kiribati	1.00758	0.87411	1.11334	0.84867	0.8655	0.64957	1.46996	1.26397												
Nauru	0.93587	0.5202	0.93855	1.09093	0.40513	1.12539	1.01033	1.55532	0.18586											
Niue	1.01481	0.93194	1.09947	0.91147	1.19741	0.28473	1.44234	0.9437	0.60542	1.08785										
Raratonga	1.01469	0.98202	0.99848	0.98147	1.24207	0.34524	1.51064	1.10277	0.7263	1.24653	0.11658									
West Samoa	0.97573	0.99285	0.95743	0.68616	0.954	0.35189	1.49636	0.93635	0.77486	1.26295	0.19794	0.14495								
North China	2.23058	1.82364	1.94243	2.84411	2.11521	1.9825	2.21571	7.3238	1.44633	1.42969	2.536	2.38533	2.22277							
South China	2.40141	3.15763	1.29088	1.06091	2.31323	1.83742	1.21264	2.13328	1.97206	2.14507	1.80667	1.77885	1.45484	0.83969						
Java	1.96473	3.35729	1.4399	1.05242	2.06784	1.43268	1.47377	2.29429	2.15186	1.65682	1.25332	1.34442	0.93177	1.55734	0.50101					
Kimberley	0.87344	1.02773	0.54603	0.84174	1.04485	0.82455	1.09257	1.05218	1.44658	3.15221	1.75631	1.51489	1.18364	2.65556	2.78369	3.45829				
Groote Eylandt	0.5432	0.60628	0.41143	0.41669	0.83523	0.30191	0.7688	0.73375	0.95793	2.0759	0.76685	0.68859	0.53743	1.82388	2.23393	2.52522	0.25677			
Yuendumu	0.47683	0.56498	0.62723	0.4097	0.69683	0.47484	1.04477	1.0949	0.90469	2.79108	0.96851	0.97019	0.77343	2.73381	2.79765	3.60983	0.15153	0.29191		
Cape York	0.59233	0.63069	0.36923	0.52497	0.94612	0.45185	0.68782	0.92721	1.28004	1.98706	1.26543	1.22211	0.99901	1.61376	1.76839	2.08119	0.18759	0.09332	0.24471	

Table 5.11 Genetic distance matrix comparing the study populations with other regional populations

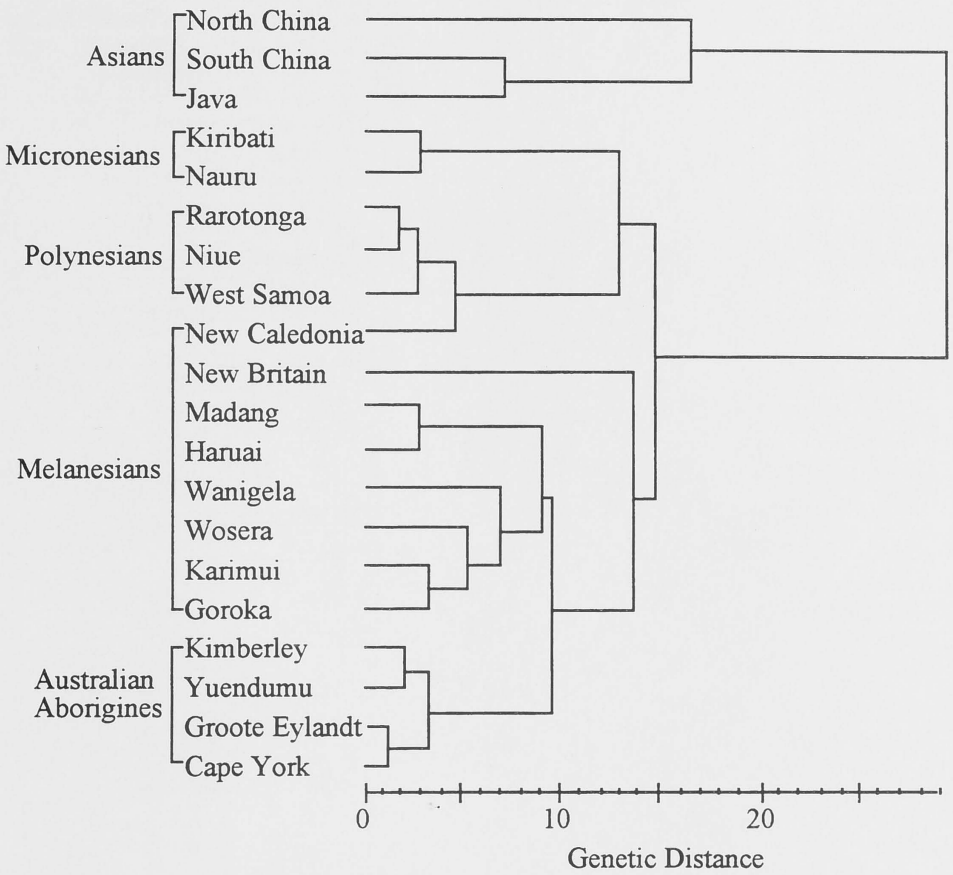


Figure 5.1 Dendrogram comparing the study populations with other regional populations based on HLA-A gene frequencies and B-C haplotypes

Chapter 6 Discussion

6.1 Introduction

High resolution typing enables the discrimination of many HLA subtypes previously undetectable by serology. A total of thirty-six alleles were detected in this study compared to the twenty previously detected using serological techniques. DNA typing was also able to resolve many haplotypes previously unrecognisable by serology, thereby providing a more accurate estimate of linkage disequilibrium between the three class I loci. DNA typing also showed that Melanesian populations have many unique HLA variants, providing new information about their origins and interrelationships. Because DNA typing is able to detect even single nucleotide differences that may be functionally significant, the results of this study may also have important implications for transplantation and studies of disease associations and natural selection.

Although Melanesians and Australian Aborigines have significantly differentiated from each other, this study provides further evidence that they may once have shared a common gene pool. The study also indicates that there was more than one pre-Austronesian movement of people into Melanesia that did not reach Australia. More recent population movement into Melanesia is shown which may have been associated with the movement of proto-Austronesians into Oceania or later migration from Southeast Asia. Some Polynesian and Micronesian backmigration is identified.

6.2 General Features of HLA in Melanesia

6.2.1 Lack of A2

HLA-A2, a common antigen in populations throughout the world, is virtually absent in all study populations despite the fact that the populations have a diverse range of backgrounds involving multiple origins over a long period of time. As

HLA-A2 is one of the most common HLA antigens in all other populations throughout the world, it is likely to have been carried into Melanesia by each successive wave of migration. Almost none, however, was detected in contemporary Melanesians suggesting that its absence is not by chance but could be due to selective pressure from unfavourable environmental factors. The sporadically detected A*0201 and A*0206 in three of the study populations are likely to be the result of recent admixture with Caucasians and Polynesians as indicated by their associated B alleles.

Except for the lack of A2, the major A alleles detected in the study populations, A*1101, A*2402 and A*3401, are the same as those detected in other regional populations including Australian Aborigines, Polynesians and Micronesians. Each of these alleles represents one of the five allele branches or families found in populations throughout the world (Lawlor et al. 1991). These alleles are likely to have been retained by balancing selection since members of different allele families tend to be structurally, and therefore functionally, more divergent than alleles from the same family. As a result, the limited repertoire of this locus is able to provide more effective immunological protection to the population.

6.2.2 B13

All the B13 detected in the study populations were the B*1301 subtype which was found in significant linkage disequilibrium with three Cw alleles: Cw*0304; Cw*0401 and Cw*1203. B*1301-Cw*0401 is likely to be the original haplotype as it was found in most study populations and is common in Australian Aborigines. In contrast, B*1301-Cw*0304 was found in two coastal/island populations, Madang and Rabaul, and was also found in Wosera. This haplotype was commonly found in Chinese but not in other regional populations suggesting that it may represent a more recent pre-Austronesian wave of migration. Alternatively it could have been generated locally. Finally, B*1301-Cw*1203, found in Rabaul and Madang, is also detected in Polynesians suggesting that it could be a locally generated haplotype that spread into Polynesia. However, it is

more likely to have spread by back-migration from Polynesia because Cw*1203 is a common allele in other Oceanic populations where it is in association with B*3901, whereas it was only found sporadically in coastal and island Melanesians. However, Cw*1203 detected in these populations is invariably associated with B*3901 but not B*1301.

6.2.3 Heterogeneity of B15

Four members of the B15 family: B*1506, B*1521, B*1525 and B*1536 were detected in this study. Except for B*1536, previously named B*15MD, these alleles are commonly found in other regional populations (Gao et al. 1997). B*1536, however, is likely to have a local origin because it has a restricted distribution and is only detected in Madang and has a much lower frequency in the Haruai suggesting that it may have originated in the Madang region.

Of the remaining alleles, B*1521 and B*1525 are likely to be older because they are commonly found in Australian Aborigines (Lienert et al. 1995) as well as Javanese (Gao et al. 1997). In fact B*1525 was only detected in non-Austronesian-speaking inland populations further supporting a closer link with Australian Aborigines, whereas B*1521 is found in other Asian populations also (Lin et al. 1996). B*1506 is likely to have been introduced later because it was detected throughout the Melanesian study populations and is common in other Pacific populations but absent in Australian Aborigines (Gao et al. 1997).

These putative relationships are further supported by their haplotype associations. For instance B*1525 is found in association with Cw*0403 in the Haruai and Goroka as in Australian Aborigines further supporting an ancient link between Australian Aborigines and earlier mainland New Guinea settlers. Similarly, an association between B*1521 and A*3401 detected in the Haruai has also been found in Australian Aborigines and Asians (Lin et al. 1996), whereas B*1506 found in Melanesia and Micronesia has the same haplotype association (Gao et al. 1997).

6.2.4 B*2704

The high prevalence of B27 in mainland New Guinea populations has been well documented in previous studies (Bhatia et al. 1988). In this study almost all the B27 detected in Melanesians was the Asian subtype, B*2704, clearly indicating an Asian source region for these populations. B*2704 was detected at unusually high frequencies in all mainland study populations but not in island Melanesians or other regional populations. It is likely that the antigen was carried by successive waves of people moving into Melanesia at similar low frequencies as are in contemporary Asian populations (1-5%). The subsequent enrichment of this antigen and its maintenance at high frequencies in all mainland New Guinea populations, despite their diverse origins, suggest that this antigen may have been selected by unidentified environmental factors. The association of B*2704 with ankylosing spondylitis is well known (Lopez-Larrea et al. 1995, Gonzalez-Roces et al. 1997), but this disease is not found in New Guinea.

The distribution of B*2704 associated haplotypes in these populations suggests that inland New Guinea was colonised by at least two different groups of people. Goroka may represent an earlier wave of migration than the other study populations because B*2704-Cw*0102 was the only haplotype detected there and it was not detected in other study populations. In contrast, B*2704-Cw*12022, a typical Asian haplotype, was found in all other study populations except Goroka, indicating a more recent movement from Asia to Melanesia. The highly confined distribution of the B*2704-Cw*0304 haplotype in the Karimui indicates this haplotype may have been locally generated from the B*2704-Cw*12022 haplotype which was also detected there.

6.2.5 B*4001

The distribution of B*4001 haplotypes in the study populations suggests that there have been at least three waves of migration into or through Melanesia. The

first of these links the non-Austronesian-speaking study populations, and Rabaul, with Australian Aborigines as B*4001-Cw*0303 was detected in these populations but is not detected in other regional populations. Later migration from mainland Asia either between the formation of the Torres Strait and before the arrival of the Austronesians or with the Austronesians was suggested by the presence of B*4001-Cw*0304 in coastal mainland New Guinea study populations. This haplotype is common in Caucasians, mainland Asians and West Samoans. A third wave of migration was suggested by the distribution of B*4001-Cw*0401 which is found in southern Chinese, Wanigela and New Caledonia, and throughout Micronesia and Polynesia.

6.2.6 B56

B56 is a common antigen in Oceanic populations, Australian Aborigines and Javanese (Gao et al. 1997). In this study DNA typing split this antigen into two subtypes, B*5601 and B*5602, with only two amino acid differences between them. Despite the structural similarity both subtypes were detected in all study populations and in all other regional populations. Except for B56, the rather limited class I HLA repertoire in these populations tend to retain more divergent alleles of different serological groups. Comparison of the three-dimensional structure of B56 reveals that one of the two amino acid differences, a change from tryptophan to leucine at position number 95, occurs in the ninth peptide binding environment (or pocket) which is an important peptide anchoring site. The other, a change from threonine to arginine at position number 97 affects five of the nine binding environments including the ninth. The large difference in size, and in the latter, charge, of these amino acids as well as their strategic placement indicate that the two subtypes have significant functional differences, which may explain the maintenance of both subtypes in all these populations.

6.2.7 Distribution of HLA B-C haplotypes

HLA B-C haplotypes were distributed in three broad patterns suggesting that Melanesia was colonised by at least three different groups of people. The first, which is likely to be the oldest, comprises a group of haplotypes found only in Melanesians and Australian Aborigines supporting the hypothesis that they arose from a common gene pool. Later pre-Austronesian population movement is indicated by low frequencies of several haplotypes in coastal mainland populations and New Britain that were also found in either Chinese or Javanese. Finally, several B-C haplotypes were identified that were commonly found in Micronesia, Polynesia and Southeast Asia that may have been associated with the movement of proto-Polynesians into the region or later movement from Southeast Asia or be the result of backmigration from Polynesia or Micronesia. These patterns of distribution of B-C haplotypes are similar to those identified by Kirk (1980) based on blood groups, red cell enzymes and serum proteins.

6.3 Austronesian and non-Austronesian-speaking Populations

It has been acknowledged that Austronesian and non-Austronesian language groups represent different waves of migration. It is generally agreed that non-Austronesian-speaking populations may represent the descendants of earlier waves of migration. Previous studies have shown that these populations share many features in common with Australian Aborigines including unique class II HLA features (Gao et al. 1992a, 1992b), α and β globin data (Roberts-Thomson et al. 1996, Liu et al. 1997) which are shared between New Guinea highlanders and Australian Aborigines. The findings support this proposed relationship as both populations share a similar 'core' range of alleles which are a subset of those commonly detected in Southeast Asian and Oceanic populations as well as many haplotypes not detected in other regional populations.

The study compared four non-Austronesian-speaking populations including Wosera, a lowland Sepik population, with four mixed/Austronesian-speaking

populations derived from coastal mainland and island regions. Although both groups shared the same 'core' of HLA alleles and haplotypes, a number of additional alleles were detected in the Austronesian-speaking coastal/island populations that were either not, or only sporadically, detected further inland. These alleles have been commonly found in Southeast Asian and/or other Pacific populations suggesting that they may have been introduced into Melanesia more recently than the 'core' HLA alleles. Their distribution throughout the Pacific suggests they may have been introduced by the Austronesians, or by later population movement from Southeast Asia or through backmigration from Micronesia and Polynesia. The detection of these coastal Austronesian markers on an all or nothing basis was possible because the study populations are well differentiated and because the fine resolution of DNA typing techniques enables the detection of a wider range of HLA polymorphism. Previously used gene markers, such as Gm, have only a few alleles so analysis was based on gene frequencies, but these can vary widely between generations in small populations and are therefore unreliable.

6.4 Populations from the Highlands, Highlands Fringe and Sepik

The HLA class I profiles split the inland study populations into three groups comprising: Goroka; Haruai and Wosera; and the Karimui, suggesting that the inland regions of New Guinea were peopled by at least three different groups of people. Goroka, a highland population, is likely to be the oldest of these groups because it is most similar to Australian Aborigines. This is in accordance with a previous study of class II HLA genes in another eastern highlands population which shared unique features with Australian Aborigines but not with other Melanesian populations (Gao et al. 1992a, 1992b). The Haruai and Wosera also share features with Australian Aborigines but have many differences from Gorokans and have a less restricted range of alleles suggesting that they are probably the descendants of a later wave of pre-Austronesian migration which became differentiated by genetic drift over a long period of isolation. The

Karimui may represent the descendants of a separate wave of colonisation as their class I HLA profile was unlike that of the other study populations.

The present study supports the proposal that there has been very limited genetic exchange between highlanders and other populations for a long period of time. The data showed that Goroka was well differentiated from the other study populations and had the most restricted range of alleles and haplotypes of any mainland study population as well as some unique haplotypes. Eastern highland populations, with a few exceptions such as the Anga, are relatively homogeneous in terms of serological typing of class I HLA (Smith et al. 1994, Bhatia et al. 1995). Eastern highlanders show considerable difference from coastal or island Melanesians based on class II HLA data (Gao et al. 1992a). This trend is also seen using multivariate analysis of polymorphic traits (Kirk 1982), skeletal analysis (Green 1990:368) and the absence of genetic markers such as the 9 bp mtDNA deletion, the thalassaemias and glucose-6-phosphate dehydrogenase deficiency from highlanders although the latter is more likely to be due to malarial selection.

Although Goroka appears to have been very isolated it shares B*5601-Cw*0702 with Madang. This shared haplotype may reflect traffic between the two populations although it may have been relatively minor. Highland trade with the north coast has been well documented (White 1972:96, Hughes 1977) and Goroka lies on the Highlands Highway which travels down to the coast. As Cw*0702 was detected at much higher frequencies in Madang than Goroka, the haplotype is more likely to have originated there.

DNA typing showed the Haruai were a typical non-Austronesian population that had the same alleles and haplotypes as other inland study populations. Interestingly, they shared two B-C haplotypes and one A-C haplotype with Australian Aborigines that were not detected in other Melanesian study populations. This distribution supports the suggestion that Yuat stock populations were colonised independently to the eastern highlands (Serjeantson

1989b). The Haruai share B*1536-Cw*0102 with Madang, which as discussed is likely to have originated in the Madang region. The Haruai language has heavy lexical borrowing from the Kobon, the population located immediately to their east, who originated in the Madang region (Comrie 1988). Any gene flow between Madang and the Haruai, however, is likely to be indirect because several unique haplotypes were detected in the Haruai suggesting that they have been isolated from the other study populations, including Madang, for a relatively long period of time.

Although Wosera had a similar profile to the Haruai it showed the influence of a low level of genetic inflow from coastal regions. The similarity in HLA profiles between Wosera and the Haruai suggests that they may have evolved from a similar founding population which later evolved differently due to long term isolation which led to different gene frequencies due to genetic drift. Gene flow from the coast is suggested by the statistically significant levels of B*1301-Cw*0304 and B*4002-Cw*0702 found in Wosera. Their distribution, however, suggests that they may be the result of different waves of migration. The former is likely to have been introduced along the north coast from mainland Asia and subsequently carried inland before the arrival of the Austronesians as it is commonly seen in Chinese but not in other regional populations. The latter was found in northern coastal/island study populations and was also found in Australian Aborigines but not in other regional populations. In both cases the possibility cannot be ruled out that the haplotypes were locally generated.

This study provided some new information about the origins of the Karimui population although a clearer answer to this question requires further study. Although the Karimui population share several haplotypes with Australian Aborigines their profile was markedly different from Gorokans suggesting that they are the descendants of a different colonising event. For instance, they were the only inland study population with no B*1525 a common antigen in Australian Aborigines. Comparison of the B*2704 associations present in the Karimui population with those found in other study populations including Goroka suggest

that the Karimui population are the descendants of a later colonising event. Although the Karimui show very little gene input from the coast, B*3901-Cw*0702, a common haplotype in coastal and island Melanesians, Southeast Asians and other Pacific populations, was detected in three individuals suggesting that it might have been carried into the population from the coast. Gm data for Pawaia and Anga speakers (Steinberg et al. 1972) also suggested there had been some Austronesian gene flow into these populations (Curtain et al. 1976). It would be interesting to compare these results with the class I HLA profile of Gulf populations in future studies.

Inland New Guinea was probably colonised by at least three different founding groups as the class I HLA profiles for the inland study populations fell into three groups comprising: Goroka; the Haruai and Wosera; and the Karimui. These populations are likely to have been isolated from each other for a long period of time because they were well differentiated from each other. Some populations, such as the Karimui, showed a low level of genetic exchange with coastal groups.

6.5 Coastal/Island Melanesian Population Affinities

The coastal/island Melanesian populations included in this study are Austronesian-speaking populations (Kirk 1982, Rhoads 1983, Bhatia et al. 1995). The region inhabited by three of these populations was peopled long before the migration of Austronesians into Melanesia whereas New Caledonia is widely believed to have been founded by Austronesians moving further east into the Pacific (Spriggs 1997: 40, 143).

Coastal and island populations are more open to external genetic influences by their very nature. In terms of HLA polymorphisms, coastal/island populations had many 'additional' alleles that were not found, or only sporadically detected in inland New Guinea. The high frequency of many of these alleles in Southeast Asians and other Oceanic populations indicates that they are likely to have been introduced by the proto-Polynesians, although they could also have been

introduced by subsequent migrations from Southeast Asia, by traders, or by Polynesian or Micronesian backmigration.

The pre-Austronesian prehistory of Madang is reflected in the HLA profile. Madang had the same 'core' of HLA alleles and haplotypes as inland populations overlayed by two other groups of haplotypes suggesting at least two more recent waves of migration. The first of these, from mainland Asia, is likely to have occurred after the formation of the Torres Strait but before the arrival of the Austronesians as suggested by the distribution of two B-C haplotypes, B*1301-Cw*0304 and B*2704-Cw*12022. The second is likely to be more recent because this set of B-C haplotypes are also detected in other Pacific populations. They were: B*3901-Cw*1203, B*4801-Cw*0801 and B*5601-Cw*0702. Madang had two unique A-B haplotypes and a unique A-C haplotype suggesting that it has been isolated from the other study populations.

Wanigela people showed a much greater degree of 'Austronesian' admixture than Madang. Although they shared the same 'core' class I HLA alleles and haplotypes they also shared a set of B-C haplotypes with mainland Asians and coastal Melanesians that are not found in other Oceanic populations. Many alleles, comprising A*2407, B*2706, B*3505, B*3901 and B*3906, and associated haplotypes commonly detected in Southeast Asian and other Oceanic populations, particularly Micronesians, were detected at Wanigela suggesting that they may have been introduced by Austronesian-speaking people up to 1,800 years before present when red-slip pottery first appears along the southern Papuan tip. Micronesian backmigration to Wanigela is suggested as A*2407-B*3505, a common Micronesian haplotype, was also detected there. Although this haplotype is occasionally found in Javanese, the alleles involved are usually found in a different association there. Low frequencies of Polynesian markers, A*0206 and B*5502, were also detected suggesting some Polynesian backmigration as well.

Rabaul has the most restricted set of alleles and haplotypes of any study population and lacks several 'core' alleles, such as B15 and B27, as well as most of the haplotypes commonly detected in other study populations which may be attributed to founder effects followed by long term isolation, genetic drift and selection. On the other hand, this population has many coastal/Austronesian alleles. Considering the geographical location of the Bismarck Archipelago on the route of Austronesian migrations, it is not surprising to see a rather significant contribution from the Austronesian gene pool.

Rabaul is the only study population with B*3903. This allele was found in Amerindians (Watkins et al. 1992) but not in other populations. Its distribution may reflect the remote common ancestry shared by the two groups of people. In previous studies of HLA class II genes unique features were also found to be shared by Amerindians and Pacific Islanders (Gao and Serjeantson 1992b). Another possibility is that B*3903 evolved independently in both populations as there is only a single nucleotide difference between B*3903 and B*3901, an allele commonly detected in the Pacific.

B*4010 was detected in coastal and island study populations and other populations in the Pacific. It was found with significant linkage with two Cw alleles. One of these, B*4010-Cw*0403, was found in all coastal and island study populations and Wosera. The other, B*4010-Cw*0702, was found only in Rabaul suggesting that this haplotype may have a local origin. Admixture with Micronesians or Southeast Asians is suggested by the presence of some unique Micronesian/Javanese markers such as A*2407 and B*3505.

New Caledonians shared the same 'core' of HLA alleles and haplotypes as the other Melanesian study populations but had more coastal alleles and haplotypes than any other study population. Although class I HLA data cluster New Caledonians with Polynesians and Micronesians, phylogenetic analysis based on class II HLA data cluster New Caledonians with other Melanesian populations well differentiated from Polynesians and Micronesians (Gao et al. 1992a). Pooled

class I and II HLA data also clearly align New Caledonians with Melanesians rather than Polynesians or Micronesians. The difference in these dendrograms may reflect different selective pressures on class I and II HLA alleles. Other genetic data such as unique allele distributions and multivariate analysis of polymorphisms (Kirk 1980) as well as skeletal analysis (Pietrusewsky 1984) also differentiate New Caledonians from Polynesians showing they are more closely related to other Melanesian populations.

The low frequencies of some common Polynesian alleles, such as B*5502 and B*3901, detected in New Caledonians support the suggestion based on archaeological, historical and linguistic evidence, that some Polynesian backmigration has occurred. Caucasian admixture is evident with the presence of typical Caucasian alleles in many individuals such as A*2501, A*6802, B*0702 and B*1401. New Caledonia has been a French colony since 1853 (Kircher 1986).

This study shows that coastal/island Melanesian populations shared common ancestral roots with inland populations that were later overlaid by subsequent population movements into the Pacific from Asia. Based on HLA data, two movements were detected. The first from mainland Asia along the mainland New Guinea coast and the latter from Southeast Asia spreading through the coastal regions of the mainland and island Melanesia into Micronesia and Polynesia. HLA data also show that some backmigration from Micronesia and Polynesia has occurred.

6.6 Has Malaria Acted Selectively on class I HLA in Melanesia?

A strong relationship between HLA and malaria has been reported from a large case-control study of children with severe malaria in The Gambia (Hill et al. 1991) suggesting that B*5301 may provide protection against the disease. In another report a reverse immunogenetics approach led to the identification of B*1513 as a potential protective allele in Malaysian aborigines (Hirayama et al. 1996). In the

present study there were four alleles that potentially bind the same peptides as B*5301. These were: B*3505, B*3901, B*3903 and B*5602.

B*5602 is the only one of these alleles found in all New Guinea populations. With the exception of the Haruai, it was found at frequencies inversely proportional to malarial endemicity in the study populations and other regional populations. Highlanders, however, are extremely susceptible to malaria when they travel to lowland areas. The Haruai have about the same gene frequency as Gorokans but are the only study population with high levels of hyperreactive malarious splenomegaly, a malaria associated disease known to have an association with an excess of DR2 (Bhatia and Crane 1985). This study was unable to distinguish whether the three alleles only detected in coastal populations confer resistance to malaria. If such association exists, however, it is likely to be weak.

Final Discussion

New Guinea's long and complex history is reflected in its extraordinary heterogeneity compared to other Pacific populations as shown by evidence from archaeology, linguistics and biological anthropology. New Guinea populations are likely to share a common ancestry with Australian Aborigines but have later differentiated from them due to long term isolation and the influence of multiple waves of migration from Southeast and mainland Asia that did not reach Australia. Of these most is known about the movement of Austronesian-speaking populations through Melanesia further into the Pacific.

Archaeological evidence suggests that people have been in New Guinea for at least 40-60,000 years (Spriggs 1997: 39). Until about 7,000 years ago, New Guinea and Australia comprised a single land mass, Sahul (Chappell 1976). The probable remote common ancestry of contemporary Melanesians and Aboriginal Australians is reflected in the restricted distribution of several unique genetic markers in these populations including some serum proteins (Kirk 1980), class I and II HLA (Gao et al. 1992a 1992b), α and β globin haplotypes (Roberts-Thomson 1996, Liu et al. 1997). Today, however, they are culturally, linguistically and anatomically well differentiated.

Contemporary Melanesian populations are very heterogeneous suggesting that there may have been multiple waves of migration into the region over a long period of time. This heterogeneity is reflected in the enormous linguistic diversity in the area - more than 20 percent of the world's languages are spoken in New Guinea alone. These populations are also extremely culturally and genetically varied. The dichotomy between lowland and higher altitude New Guinea populations is well established. At the time of European contact there were two concentrations of people living in New Guinea - those living at altitudes greater than 1,300 m and those living on the coast penetrating inland to an altitude of 600

m (Parkinson 1974). These populations were linguistically, culturally and morphologically well differentiated.

Highlanders are likely to represent the descendants of earlier waves of migration into New Guinea as they share several unique genetic features with Australian Aborigines that are not found in other populations. These include similarities in class I and II HLA (Gao et al. 1992a, 1992b), α and β globin haplotypes (Roberts-Thomson et al. 1996, Liu et al. 1997). These populations are likely to have been isolated from other regions for some time as suggested by skeletal analysis (Green 1990) and the absence of many polymorphisms commonly detected in other populations. Highland populations to the west of the Strickland Gorge, however, are likely to have been somewhat less isolated based on class I HLA serology (Smith et al. 1994) and the presence of low frequencies of ovalocytosis and glucose-6-phosphate dehydrogenase deficiency (Holt 1981, Schuurkamp et al. 1989).

Northern highlands fringe and Sepik populations are likely to represent the descendants of a more recent colonising event which later differentiated due to long term isolation. Of these, Sepik populations show more gene flow from the coast and have the highest level of malarial endemicity in New Guinea which is reflected in the high frequency and wide range of malarial associated red blood cell polymorphisms found there (Wagner 1996). In contrast, the southern highlands fringe has probably been peopled by a separate group of people. Oral tradition (Warrillow 1978), linguistic (MacDonald 1973) and cultural evidence and red blood cell polymorphisms (Russell et al. 1971) suggest that they may have originated in the Gulf area. Some admixture with the neighbouring Daribi and Angan populations, however, is likely to have occurred based on class I HLA serology (Bhatia et al. 1988) and marriage patterns (Nurse 1981).

Mainland coastal and Bismarck Archipelago populations are more heterogeneous and show the influence of population movement along the coast that did not reach inland. Population movement from Southeast and mainland Asia is suggested by

the distributions of several unique red blood cell enzymes and serum proteins, ovalocytosis, and class I and II HLA polymorphisms shared by coastal/island Melanesian populations that are not found in other regional populations. Their distribution suggests that they may have been introduced between the formation of the Torres Strait and before the arrival of Austronesian-speaking populations into the region.

Of the continued genetic input from outside sources, most is known about the movement of Austronesian-speaking populations through Melanesia into the Pacific. Archaeology has shown that these people were associated with a distinct cultural complex and linguistic evidence suggests that these people originated in Taiwan before moving through Melanesia via the Bismarck Archipelago to occupy Remote Oceania. Examination of skeletal remains of these people has consistently placed them closer to contemporary Southeast Asian and Polynesian populations than Melanesians (Pietrusewsky 1975, 1997, Kirch et al. 1989). Although no unique Austronesian genetic markers have been identified, Austronesian-speaking Melanesian populations share many characteristics with Southeast Asians, Polynesians and Micronesians that are not found, or only sporadically detected, in inland New Guinea. These include a range of red blood cell enzymes and serum proteins, class I and II HLA and the clinal distribution of 9 bp mtDNA deletion.

Backmigration to the Bismarck Archipelago and coastal mainland New Guinea is suggested by the clinal distribution of Hb*Tongariki and $-\alpha^{3.7}$globin and their likely origin in northern Vanuatu (Higgs et al. 1984, Hill et al. 1985, Flint et al. 1986, Yenchitsomanus et al. 1986a) and its clinal distribution northward. Oral tradition, archaeology, and linguistic evidence suggest that backmigration has also occurred from Polynesia (Spriggs 1997). Captain Cook's journals record the presence of Tongan canoes along the entire east coast of the main island of New Caledonia. The distribution of low frequencies of class I HLA haplotypes commonly found in Polynesia, along the New Guinea coastline and in the Bismarck Archipelago strongly supports these assertions.

Melanesian/Micronesian interrelationships are somewhat less well documented although there appears to have been some cultural diffusion from Micronesia (Intoh 1996). The detection of several class I HLA alleles and haplotypes commonly found in Micronesians at Wanigela invites speculation about the relationship of these genes to the introduction of red-slip pottery in this area about 1,800 years ago followed by later cultural change attributed to a second wave of Austronesian-speaking people.

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Appendix 1: Oligonucleotide Probes - hybridisation patterns

B locus